EVALUATING POTENTIAL AGE STRUCTURES FOR THREE ALASKA CRUSTACEAN SPECIES

By

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Abstract

Banding patterns are observed in calcified structures of red king crab (*Paralithodes camtschaticus*), snow crab (*Chionoecetes opilio*), and spot shrimp (*Pandalus platyceros*). Recent research supports an age determination method based on these banding patterns; however, processing methodologies for these structures have not been established. Further, species-specific evidence is needed to determine whether these patterns indicate actual age or growth. The objectives of this thesis are to: (1) describe optimal species-specific methods for producing and evaluating band counts for red king crab, snow crab, and spot shrimp; and (2) use differences in shell condition to test whether band counts indicate age for snow crab. For each species, we comprehensively thin-sectioned structures, evaluated each section for banding pattern presence (readability), and developed band count criteria. To address objective 1, we used generalized additive models to describe readability across structures to find the location that optimizes the production of readable sections. For objective 2, we used a one-way ANOVA to compare band count and endocuticle measurements among shell conditions in snow crab. Results indicated preferred structures, locations, section orientation, and thickness. Results also indicated that there is no relationship between band count and shell condition for terminally molted snow crab. These results describe optimal methods for processing crustacean structures and suggest that the potential age structures may not continue to produce bands after terminal molt in the case of snow crab. Further evaluation is needed to validate potential age relationships and the use of this technique for age estimation.

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General Introduction

Age determination of fishes is commonly accomplished using scales or otoliths, structures that record and express growth history via visible patterns. Growth patterns appear as bipartite bands and are formed by the accumulation of structural material at differing rates (i.e. fast growth in the summer followed by slow growth in the winter) (Brothers et al., 1976; Pannella, 1971). Because the varying width of bands indicate growth variability, they can be associated with environmental changes and allow researchers to identify possible causes in population characteristics (e.g., Sogard and Able, 1992). Ages derived directly from structures can help identify strong and weak cohorts, which can provide insight into the success or failure of particular populations and strengthen stock assessments (Campana, 2001).

Age determination of crustaceans is complicated because growth processes involve replacement of the chitinous exoskeleton via cycles of formation of a new cuticle and subsequent shedding of the existing rigid cuticle (e.g. molting) (Dillaman et al., 2013). Loss of the cuticle implies loss of all or most structures comprised of cuticle, leading to the conclusion that crustaceans do not retain structures capable of recording growth history continuously throughout their lifetime (Vogt, 2012). As a result, indirect methods, mainly size measurements, are used as proxies for age estimation (e.g. Zheng and Kruse, 2014).

Recent research has identified banding patterns within certain calcified structures of crustaceans presumed to be retained through the molt and has proposed a direct age determination method based on the observed patterns (Kilada et al., 2012; Leland et al., 2011). A preliminary assessment of the method confirmed the presence of banding patterns for three commercially

important Alaska crustaceans: red king crab (*Paralithodes camtschaticus*), southern Tanner crab (*Chionoecetes bairdi*), and spot shrimp (*Pandalus platyceros*) (Kilada et al., 2017). However, methods for processing the calcified structures require species-specific standardization in order to effectively compare banding patterns between individuals. In addition, there is a lack of evidence linking the banding patterns to other indicators of age for the three Alaska species. With this in mind, the goal of this thesis is to develop standardized procedures for species-specific application of this novel direct age estimation method by thin sectioning, microscopic evaluation, and age reading (banding pattern interpretation) for red king crab, snow crab (*Chionoecetes opilio*; a southern Tanner crab congener), and spot shrimp. These methods were applied in an observational study to evaluate the potential for growth band formation in male snow crab in the absence of molting.

Two chapters address the thesis goal. Chapter 1 objectives are: (1) develop and standardize species-specific procedures for sectioning calcified hard parts, (2) develop species-specific criteria for estimation of band counts, and (3) determine whether band counts are related to size classes for red king crab, snow crab, and spot shrimp. The objectives for Chapter 1 were reached by evaluating banding pattern presence and clarity (readability) among thin section orientations, thin section thickness, each of the proposed structures, and within structures among individuals; and producing standardized band counts for large and small size class individuals for each species.

Chapter 2 uses the standardized methods recommended for snow crab from Chapter 1, and the objectives are to determine whether: (1) bands are formed in the endocuticle in the absence of

molting, (2) the endocuticle grows after the terminal molt, and (3) relative differences in mean band count among shell condition groups are consistent with current understanding of age and growth. Chapter 2 objectives were reached by producing band counts and measuring endocuticle thickness from standardized thin sections for snow crab of varying shell condition, and to determine whether band counts differ among shell condition groups consistent with post-terminal molt age estimates from mark-recapture research.

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Chapter 1: Development of procedures for sectioning calcified hard parts as potential age structures for three Alaska crustacean species[1]

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Abstract

Banding patterns are present in the endocuticle of gastric mill ossicles and in the eyestalks of red king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*), and eyestalks of spot shrimp (*Pandalus platyceros)*. Recent research supports attempts to develop an age determination method based on these banding patterns; however, processing methodologies for these structures have not been established. The goal of this study is to describe optimal species-specific methods for producing and evaluating band counts for red king crab, snow crab, and spot shrimp. For each species, we comprehensively thin-sectioned structures, evaluated each section for banding pattern presence (readability), and developed criteria to produce band counts for small and large individuals of each species. We used generalized additive models to describe readability across structures to find the locations that optimize the probability of readable sections. Results indicated preferred structure, location, section orientation, and thickness for each species. In addition, we described the relationship between band count and carapace size for all three species. These results suggest optimal methods for processing crustacean structures and illustrate that the band counts may be useful for estimating growth. Further evaluation and validation are needed to authenticate this technique for age estimation.

Introduction

The United States (US) ranked third behind China and Indonesia in terms of global marine fisheries production in 2014 (FAO, 2016). Within the US, Alaska was the leading state both in terms of weight (2.54 billion tons or 58.3 % of US total) and ex-vessel value of fishery landings (US$1.6 billion or 30.1%) in 2016. Bering Sea crab fisheries generated US$303 million in 2016 (Garber-Yonts and Lee, 2017). Southeast Alaska shrimp ex-vessel value was estimated to be US$2.7 million during the 2016-2017 season (Smith and Gray, 2017). In addition to their economic value, crustaceans, such as crab and shrimp, also play vital ecological roles in benthic marine food webs where they serve as primary consumers and important prey for other invertebrates, fish, seabirds, and marine mammals (Duffy et al., 2001). As seafood, crustaceans are in high demand, but despite restrictions and regulations to sustain populations and fisheries, most of the commercially important Alaska crustacean stocks are at historically low stock sizes. Many fisheries have remained closed since the early 1980s due to conservation concerns associated with low abundance. Their economic and ecological importance and recent population trends have stimulated new research to improve stewardship of crustacean resources.

Conservation efforts to prevent continuing population declines and overfishing include stock enhancement (Persselin, 2006), ecosystem monitoring (Zador et al., 2016), and more conservative management strategies (e.g., Kruse et al., 2010). In recent decades, quantitative stock assessments have been developed for fishery management of commercially important crustaceans off Alaska based on a suite of alternative length-based approaches, depending upon level of data availability (Zheng and Kruse, 2014). Abundance estimates resulting from length-based approaches tend to be relatively imprecise, due in part to inherent variability in individual

growth histories resulting in a wide range of ages for crustaceans of any given size. Availability of routine age composition data for crab and other crustaceans would allow a transition from length-based to more precise age-structured stock assessments. Further, the ability to determine crab and shrimp age would improve estimation of fundamental life history parameters (e.g., growth, age-at-maturity, natural mortality, recruitment), which remains a longstanding critical research need.

Standard direct age determination methods for fish and invertebrates involve interpreting growth patterns within biological structures, such as bones, scales, and shells (Abele et al., 2009; Campana, 2001; Jackson, 1994; Kilada et al., 2007). Indirect age determination methods for crustaceans such as size are derived from captive observations, tag-recapture experiments, and application of modal analysis of size-frequency distributions (Hartnoll, 2001; Vogt, 2012). Research using chemical marking, observations of exuviae, and band counts in the endocuticle, suggests that the crustacean cuticle may be retained through molting and indicate age (Kilada et al., 2012; Leland et al., 2015); however, recent histological studies have shown the cuticle is not retained through molting (Becker et al., 2018; Sheridan et al., 2015). Growth band counts within the endocuticle region of eyestalks and the gastric mill correspond well with indirect estimates of age rather than the expected number of molting events for squat lobster, shrimp, crab, crayfish, and krill (Kilada and Acuña, 2015; Kilada et al., 2012; Leland et al., 2015). In a recent pilot study on Alaska red king crab (*Paralithodes camtschaticus*), southern Tanner crab *(Chionoecetes bairdi*), and spot shrimp (*Pandalus platyceros*), the endocuticle was identified, bands were observed, and retention of these structures through molting was suggested and identified as a key area for ongoing investigation in all three species (Kilada et al., 2017). Ongoing studies have

shown promising application of this method; however, a standard method for processing structures has not yet been proposed.

The goal of this study is to develop and evaluate various procedures that may be suitable to apply to endocuticle band counting in red king crab, snow crab, and spot shrimp in Alaska. To this end, we assessed variation with regards to banding pattern presence (readability) and numbers of bands (counts) among locations within a structure, among structures within a specimen, with section orientation, and between specimens of contrasting body size to define species-specific procedures for sectioning and estimating band counts. Structures evaluated include paired eyestalks for all species and, for snow and red king crab, the paired zygocardiac and pterocardiac ossicles and the uromesocardiac ossicle of the gastric mill.

Methods

Specimen Collection

Small immature (≤ 70-90 mm carapace length, CL) and large mature (140-160 mm CL) newshell intermolt red king crab were collected (N=5 per group) during the National Marine Fisheries Service (NMFS) crab and groundfish trawl survey from central Bristol Bay in the southeastern Bering Sea (Daly et al., 2015) on June 9, 2015. Small immature (40-60 mm carapace width, CW) and large mature (80-100 mm CW) newshell intermolt snow crab were collected (N=5 per group) during the same survey from central northeast Bering Sea on June 24 and July 5, 2015. Small male (20-30 mm CL) and large female (40-50 mm CL) spot shrimp were collected (N=5 per group) during ADF&G pot surveys conducted in Ernest Sound, located in southern Southeast

Alaska (Smith et al., 2014) in September 2015. All specimens were frozen at sea. Standard biological data (CL or CW and sex) were recorded for each specimen.

Structure Processing

Paired eyestalks (for crabs and shrimp) and stomachs containing the gastric mill (paired zygocardiac and pterocardiac ossicles and the uromesocardiac ossicle for crabs only) were dissected at the ADF&G Mark, Tag, Age Lab, Age Determination Unit (ADU) in Juneau, Alaska, and preserved in a 70:3:27 mixture of ethanol, glycerol, and water. Non-structural tissues and excess fluids were removed before the structures were weighed (±1 µg). Structure length and height (Fig. 1.1A) were measured with digital calipers (±0.01 mm) for each of the eyestalks and zygocardiac, pterocardiac and uromesocardiac ossicles. Next, structures were embedded in System Three® cold cure epoxy and thin-sectioned with a Buehler® IsoMet™ 5000, high-speed diamond blade precision saw, programmed to cut each section at 180 µm. Thin section thickness was measured with digital calipers (±0.01 mm). All structures were sectioned at orientations described by Leland et al. (2011) and Kilada et al. (2012). Namely, shrimp eyestalks and crab uromesocardiac ossicles were sectioned longitudinally, and crab eyestalks, zygocardiac and pterocardiac ossicles were sectioned transversely, except for structures selected for orientation comparisons trial.

A trial was run using structures from one large, haphazardly selected specimen per species to compare readability across longitudinal and transverse thin sections. The left of one paired structure (eyestalk, zygocardiac, and pterocardiac) was sectioned transversely while the right paired structure was sectioned longitudinally and the uromesocardiac ossicle was sectioned

transversely (orthogonal to reported orientations, Fig. 1.1B). Likewise, readability was compared across a range of thin section thicknesses. Variability in section thickness was due to the performance of the high-speed saw around the targeted thickness (180 μm). After sectioning, all thin sections were evaluated with a Leica DM 2000 compound microscope using transmitted and reflected light, wetted with tap water, and under magnification between 125x and 1000x. Thin section readability was based on presence and clarity of banding patterns and determined subjectively as either readable (1) or unreadable (0) (e.g. Fig. 1.2).

Band Count

Serial sections were identified by their standardized distance (ranging from 0 to 100) along the cutting axis of the structure (Fig. 1.3). Band count criteria were based on published methods (Kilada and Acuña, 2015; Leland et al., 2015) and further developed with consultation from international experts, Dr. Jesse Leland and Dr. Raouf Kilada. First, the endo-exocuticle boundary was identified as the origin. Next, the band counting occurred along the preferred axis, beginning at the origin and ending at the endo-hypocuticle boundary (Fig. 1.4A). Bands were defined as paired light and dark zones, perpendicular to the preferred axis, and continuous throughout the thin section (Fig. 1.4B). For each species, a reference collection was created containing 25 readable thin sections randomly selected from a variety of structures, including both small and large specimens. Four professional age readers from the ADU were trained to interpret banding patterns based on criteria using annotated images of five thin sections from the reference collection. To test interpretation, the four readers then independently produced band counts for the 20 remaining reference collection thin sections. Agreement among readers was used to determine repeatability (precision).

Results from cut orientation, sectioning thickness, structure, specific location, and band count assessments were used to produce a final band count for each specimen of each species. To develop a final band count estimate, primary and secondary band counts were independently produced and compared, and discrepancies were independently resolved.

Data Analyses

Structure Processing

An alpha level of 0.05 was used for all statistical tests. To determine optimal section orientation, thickness, structure and location, we compared proportions of readable structures using generalized additive and generalized additive mixed models (GAMs and GAMMs, respectively) and analysis of variance (ANOVA). Thin sections that were damaged during processing were excluded from analysis. Readability was assumed to be independent of date evaluated, which was supported by the absence of trends in readability across date. For linear models, readability data were binomial.

To determine optimal section orientation, proportions of readable sections were tabulated across structures and species. The orientation that produced the highest proportion of readable sections was reported in the results as the preferred section orientation. Proportions of readable sections for paired structures were compared to one another (left vs. right). Proportions of readable sections of the uromesocardiacs that were sectioned transversely were compared to the average proportion across all uromesocardiacs sectioned longitudinally. A model was not fit to the data due to the limited portion of readable sections.

To suggest methods to optimize sectioning thickness, the probability that a given section is readable was modeled as a function of thickness (1) with R package (Wood, 2015). Readability was not expected to increase or decrease linearly with thickness, so a GAM with a logit link was used:

$$\log\frac{p}{1-p} = \alpha + s(t), \quad (1)$$

where p is the likelihood of containing a readable section, t is the thickness of the thin section, and s is a smoothing function of thickness.

A simple ANOVA of the average readability of individual structures was used to detect differences among structures, and a chi-square test was used to compare proportions of readable sections among structures. The probability that a given section is readable was modeled as a function of distance and size class, while accounting for random differences in the proportions of readable sections among specimens or among size classes (2). A GAMM with a logit link was used:

$$\log\frac{p}{1-p} = \alpha + a_i + s_i(l), \quad (2)$$

where p is the likelihood of containing a readable section, l is the location at which the thin section occurs along the length of the structure, s_i is a smoothing function of distance for specimen i, and a_i represents a random intercept for specimen i. In addition, a model was fit to allow for different intercepts between the two size classes (fixed effects α_w) and separate smoothing functions by size class (s_w). Finally, reduced models were fit with a single intercept

and smoothing function across specimens and size classes. The importance of specimen and specimen size category effects was tested using an Akaike information criterion (AIC) comparison.

Band Count

To assess band count precision among readers, each reader's estimate was compared to the average estimate of the remaining readers. Diagnosis of paired age agreement methods described by McBride et al. (2005), illustrations (Cailliet et al., 2006; Campana et al., 1995), age-bias plots, and age difference plots were used for visual and graphical analyses using the FSA package in R (Ogle, 2018). Average percent error (APE) (Cailliet et al., 2006; Kimura and Anderl, 2005) and Chang's coefficient of variation (CV) (Campana et al., 1995) were used as indices of band count precision between readers. Bowker's, McNemar's, and Evans and Hoenig's tests of symmetry (Cailliet et al., 2006; McBride et al., 2005) were used to support visual assessment. Statistical analysis of differences in band count between specimen size classes were conducted using a one-way ANOVA model with size as the response variable and band count as the independent variable.

Results

Structure Processing

Processing structures from dissection to band count production required approximately 150 hours of time for the 30 specimens. After sectioning and initial thin section evaluation to remove broken or damaged structures, we used a total of 280 previously published orientation thin section readability assignments and 177 alternative orientation readability assignments to

calculate orientation, structure, and species-specific percentages of readable sections (Table 1.1). These values ranged from 0% to 33% readable. Because we were unable to run significance tests among the proportions readable between section orientations, any increase in the proportion readable using the alternative orientation was considered an improvement on the previously published preparation methodology. For red king and snow crab, readable proportions did not increase with longitudinal sectioning of zygocardiac and pterocardiac ossicles. However, larger proportions of readable sections were observed for longitudinally sectioned snow crab eyestalks and transversely sectioned uromesocardiac ossicles for both snow and red king crab. There were no improvements in readable proportions of spot shrimp eyestalks with transverse section orientation. Overall, the only structures that experienced improvement by sectioning at orientations alternate to previously published methods were snow and red king crab uromesocardiac ossicles and red king crab eyestalks. Although improvements resulted from the alterative orientation for crab uromesocardiac ossicles and crab eyestalks, the previously published orientation was used to process the majority of the structures; therefore, those orientations were used for the remaining analyses.

While we programmed equipment to cut each section at 180 μm, thin section thicknesses varied within each species and we used a model to identify a target thickness to optimize the production of readable thin sections. The range of thin section thickness was 40 to 730 μm, 70 to 320 μm, and 150 to 730 μm for red king crab, snow crab, and spot shrimp structures, respectively. The GAM for readability probability across thickness suggested that thickness was not a significant factor for estimating the proportion of readable sections in red king and snow crab structures ($p= 0.401$ and $p= 0.582$, respectively). However, visual inspection of the fitted models suggests

potential optimization for thickness within ranges where data are dense (approximately 160-200 μm thick, Fig. 1.5). For spot shrimp eyestalks, the proportion of readable sections was significantly associated with thin section thickness (df= 1.396, p= 0.009), with the model indicating optimal thickness to be less than 200 μm thick (Fig. 1.6).

For each crab species, proportions of readable sections did not significantly differ among structures (one-way ANOVA df= 3, F= 1.191, p= 0.312 and chi-square p= 0.309 for red king crab; one-way ANOVA df= 3, F= 0.360, p= 0.735 and chi-square p= 0.453 for snow crab) or among left and right (paired) substructures (one-way ANOVA F= 0.493, p= 0.483 and chi-square p= 0.556 for red king crab; one-way ANOVA F= 232, p= 0.678 for snow crab). Thus, the zygocardiac was selected for analysis of readability among serial sections, due to its common use in related literature. Left and right zygocardiacs were combined in the subsequent analyses. An AIC comparison of the models (Table 1.2) suggested that the best model for red king crab estimates readability across location within the zygocardiac for large and small specimens combined. The average pattern of readability across all red king crab specimens, based on a model with a single intercept and smooth function, shows a peak in the probability of finding a readable section at a location of approximately 65 (65% of the structure's total length from proximal to distal end, Fig. 1.7). For snow crab, Table 2 indicates that the best model is one that estimates separate trends in readability for small and large crab, suggesting that large crab are more likely to have readable sections on average (Fig. 1.8). The average pattern of readability across all snow crab, based on a model with a single intercept and smooth function (ΔAIC = 5, Table 1.2), shows a peak in the probability of finding a readable section at a location of approximately 35 (35% of the structure's total length from proximal to distal end, Fig. 1.9).

There was insufficient evidence to support a significant difference in readability among location within spot shrimp eyestalks (one-way ANOVA df= 197, F= 0.609, p= 0.436).

Band Count

For band count precision among independent readers, age bias plots (Fig. 1.10 and Fig. 1.11) and results of tests of symmetry (Table 1.3) suggest that pattern interpretation is generally repeatable with readable thin sections for red king crab, snow crab, and spot shrimp structures. Average percent error (APE) of a given reader against the average of the remaining readers was between 11% and 28% for all three species.

Due to low probability of producing readable thin sections for all species, analyses for band count versus body size included a total of eight red king crab (four small and four large), nine snow crab (four small and five large), and eight spot shrimp (four small and four large). Snow crab CW significantly differed among band counts (one-way ANOVA df= 7, F= 19.58, p= 0.003). Red king crab and spot shrimp CL also significantly differed among band counts (one-way ANOVA df= 6, F= 16.68, p= 0.006 and one-way ANOVA df= 6, F= 8.888, p= 0.025, respectively), although the same number of band counts were estimated for some large and small individuals (Fig. 1.12).

Discussion

This study led to development of optimal methods for processing potential age structures for red king crab, snow crab, and spot shrimp. Comprehensive evaluation of the structures limited the number of individual specimens per species. Only one specimen per species was used to perform

alternate section orientation trials, and final band counts could only be produced for 4-5 specimens per size class. Even with optimal methods, the probability of producing a readable thin section with clear banding patterns did not exceed 75% and, in most cases, was less than 50%. However, the proposed optimal methods reduce processing time significantly by refining thin section thickness, structure, and location, and future studies should be able to produce readable and comparable thin sections efficiently for the three evaluated species.

Results suggest the banding patterns contained within thin sections of eyestalks and gastric mill ossicles can be interpreted precisely, across independently produced reads. Although an APE of approximately 5.5% is acceptable for many ageing studies (Campana, 2001), our APE values between reader ranged over 11.9%-27.1%. Readers used calibration techniques before producing estimates; however, we expect precision would improve with additional experience over time.

The band count estimates from this study are generally consistent with previous age estimates derived from growth studies. Our estimates for small red king crab band counts fell within the age range (ages 3-7 years) modeled at size for red king crab by McCaughran and Powell (1977), except for the band count estimate of 11. Likewise, for large red king crab, our estimates fell within the modeled range (ages 5-15 years, also from McCaughran and Powell (1977)) except for those over 15, which are overestimated according to the model. Snow crab band count estimates were consistent with age at molt derived from size-frequency analysis (Sainte-Marie et al., 1995) for the small size class (age range 4.3-5.7 years), but our large specimens estimates were greater than the modeled range (ages 7.7-9.7 years). Our shrimp band count estimates for the small size group are within the age range reported from a Prince William Sound, Alaska,

mark-recapture study (ages 0-4 years) while our estimates for the large size group fall short of the mark-recapture range (ages 5-8 and >10 years, respectively) (Kimker et al., 1996). Additionally, with red king crab and spot shrimp, the same band count estimates were produced for some large and small individuals (Fig. 1.12), but this occurrence could be attributed to the need to improve pattern interpretation. Large size-class band count estimates from our study tend to be greater than reported expectations, but our estimates for the small size-class across all three species are within reasonable ranges. In effect, our estimates allow the possibility that the bands are indicative of age.

Whether or not the bands represent annual growth increments for these species is yet to be determined. Due to evidence of higher molt frequencies than band counts at the respective size for each species (Powell, 1967; Sainte-Marie et al., 1995), it is unlikely band counts indicate molt number or instar. Red king crab are observed to molt a minimum of 12 times by the time their carapace is 84 mm long (Powell, 1967), while our band counts for individuals under 80 mm reached 7 at the highest. Likewise, snow crab are observed to molt a minimum of 8 times by the time their carapace is 40 mm wide (Sainte-Marie et al., 1995), yet our maximum band count estimate for individuals between 40 and 60 mm was 5. Evidence of spot shrimp molt frequency is limited; however, several studies suggest spot shrimp molt more than once per year early in their growth (Kimker et al., 1996; Lowry, 2007; DFO, 2016/17), and our band counts for large shrimp, which fall short of age-at-size estimates, would further underestimate molt number.

Species- and region-specific life history events could provide corroborative insight into features of the banding patterns. For example, male snow crab growth changes with maturity, marked by

a change in the relationship between chela height and carapace width (Tamone et al., 2007). Similar to otolith growth transitions associated with the onset of sexual maturity (Francis and Horn, 1997), evidence of these changes could be used to support a minimum band count estimate. In addition, radiometric age determination using material from the endocuticle of the structures could be considered for band count age validation techniques (Ernst et al., 2005). Until robust validation of annual or sub-annual growth, the methods presented in this study are indirect and unverified for age determination.

Conclusion

With the caveat that the proportion of readable sections was low for all species, we successfully developed standardized methods for efficient and repeatable production of readable thin sections for further investigation of direct age determination. Our band count estimate findings suggest that banding patterns in the structures are related to animal size, and, therefore, may be indicators of age. However, the previously published use of this method to produce ages for management is currently thwarted by lack of validation of true absolute age and understanding of the mechanism of band formation. Use of standardized methods and direct validation of the structural bands as actual growth increments is necessary before the structures can be used to produce ages for stock assessment.

Acknowledgements

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Figures

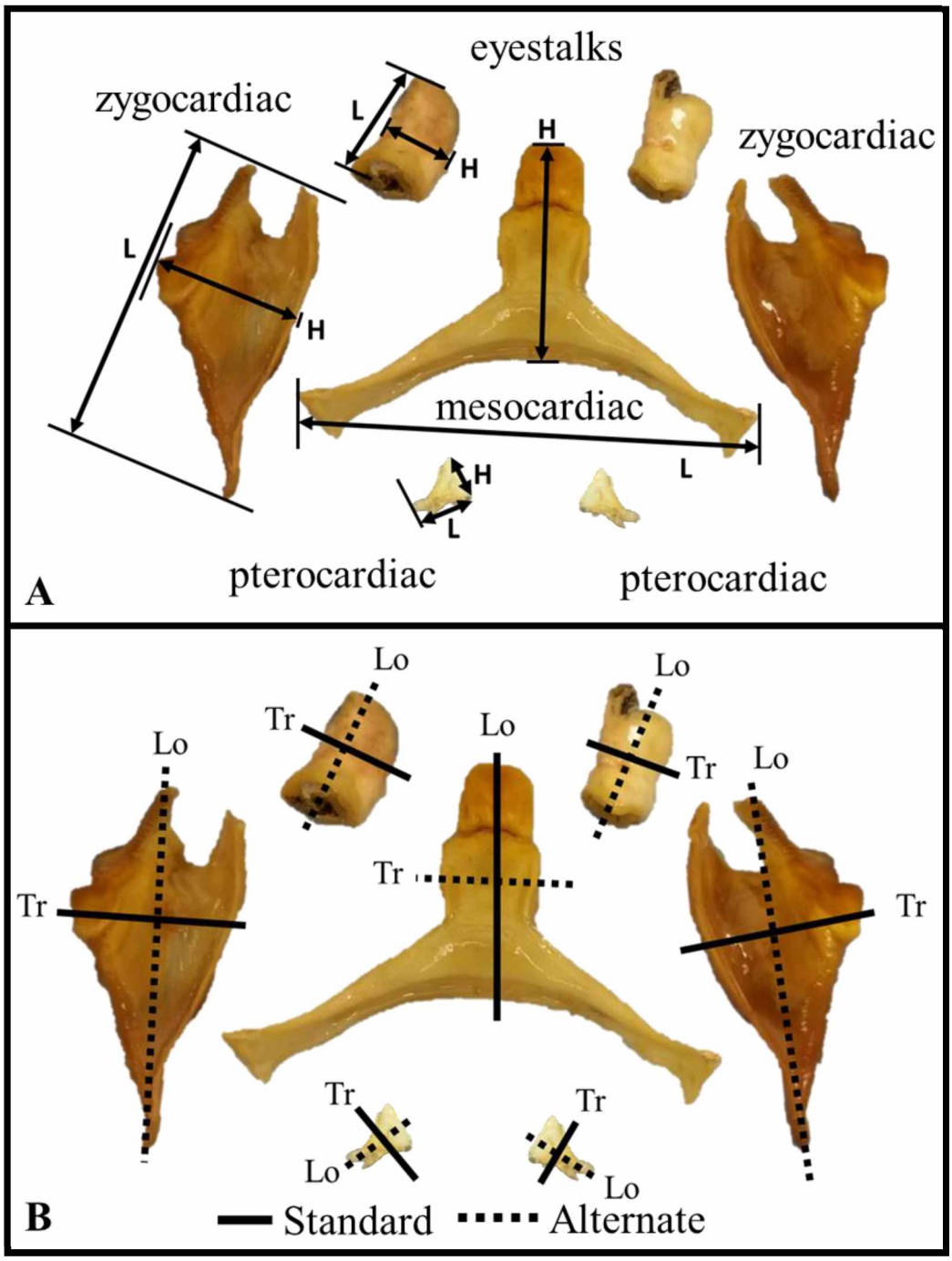

Figure 1.1. Dissected and cleaned red king crab eyestalks and gastric mill ossicles with (A) length (L) and height (H) measurements and (B) thin sectioning orientations for longitudinal (Lo) and transverse (Tr) cuts, including whether orientations were previously published or alternate for each structure.

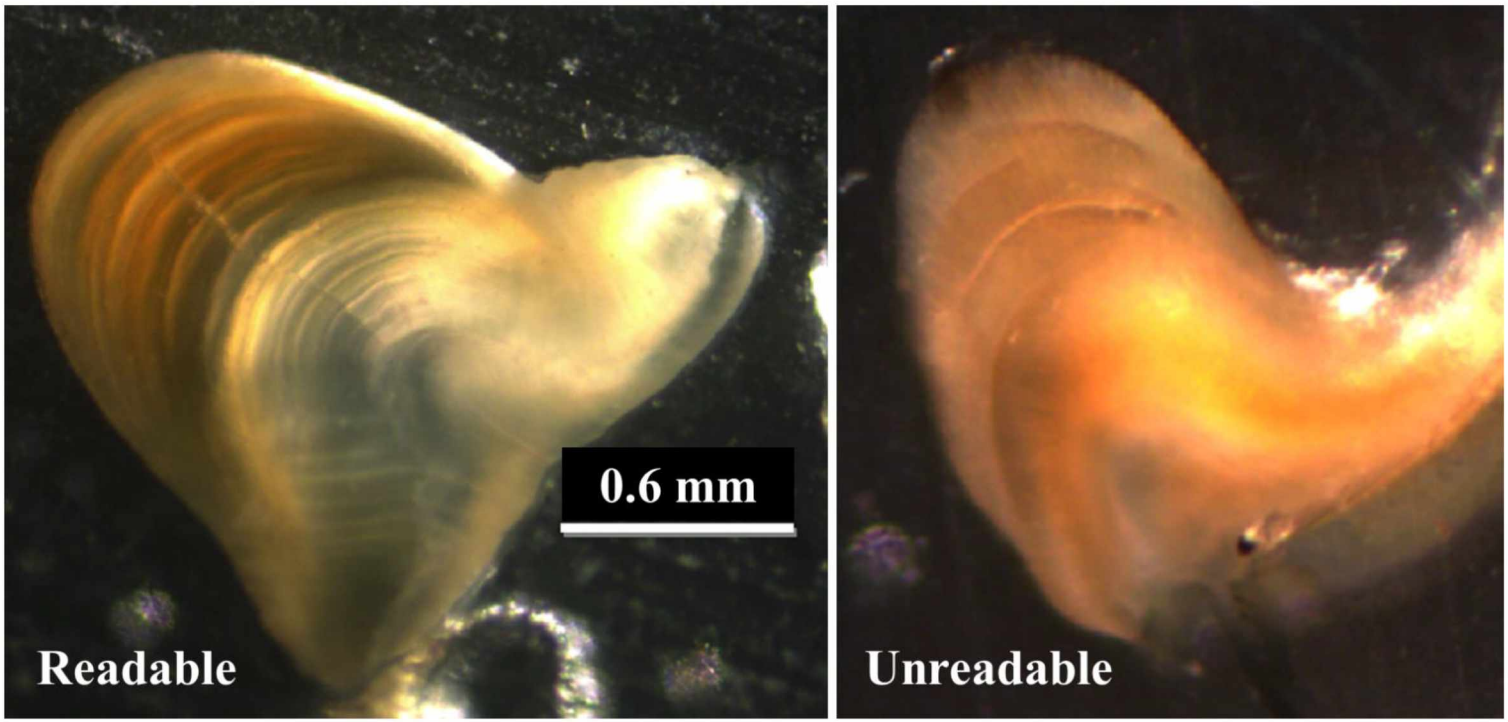

Figure 1.2. Examples of readable (left) and unreadable (right) snow crab zygocardiac thin sections.

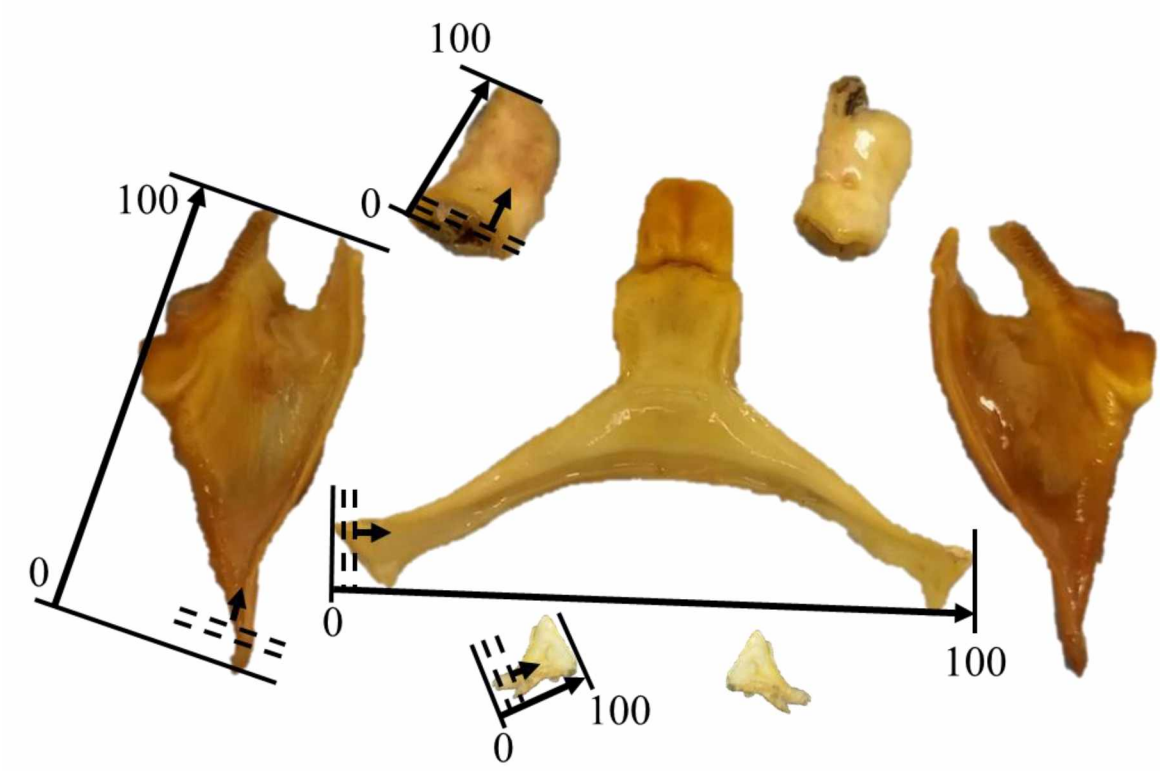

Figure 1.3. Examples of thin section location, orientation, and direction for red king crab eyestalks and gastric mill ossicles with solid line marking length converted to distance from the proximal end (0) to the distal end (100) for location and dashed lines marking orientation and direction.

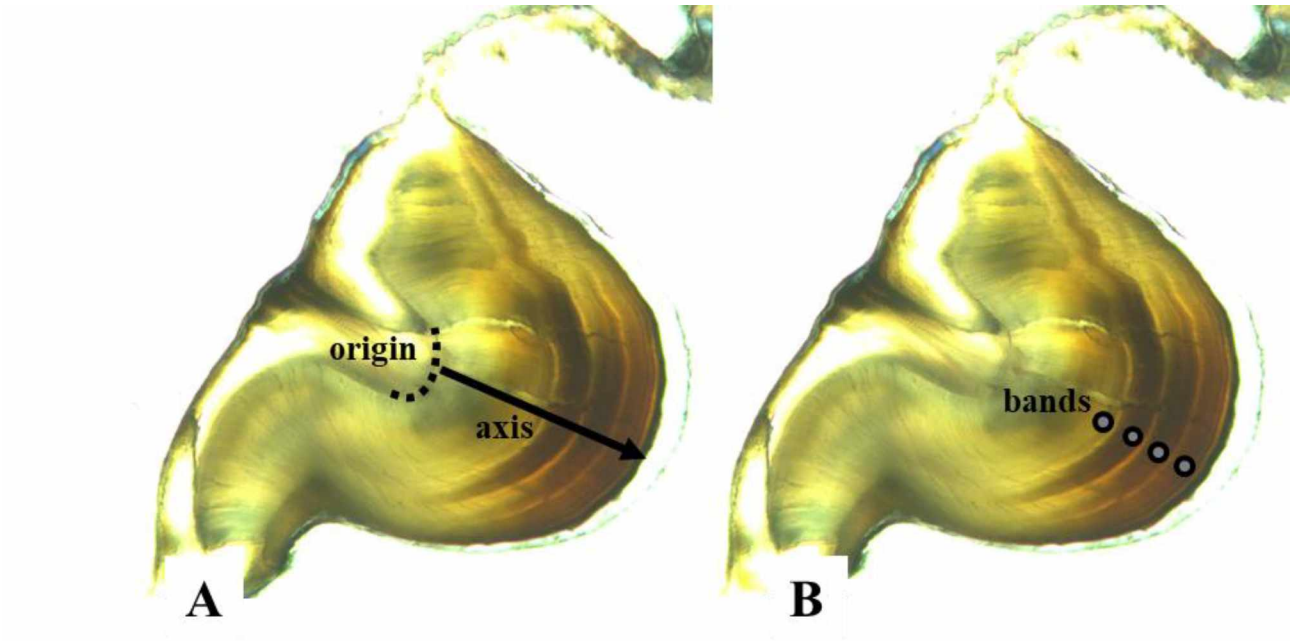

Figure 1.4. Images of a snow crab zygocardiac thin section with criteria used to produce band count estimates. The origin denotes the endo-exocuticle boundary, the preferred axis begins at the origin and ends at the endo-hypocuticle boundary (A). Bands are paired light and dark zones, perpendicular to the preferred axis, and continuous throughout the thin section (B).

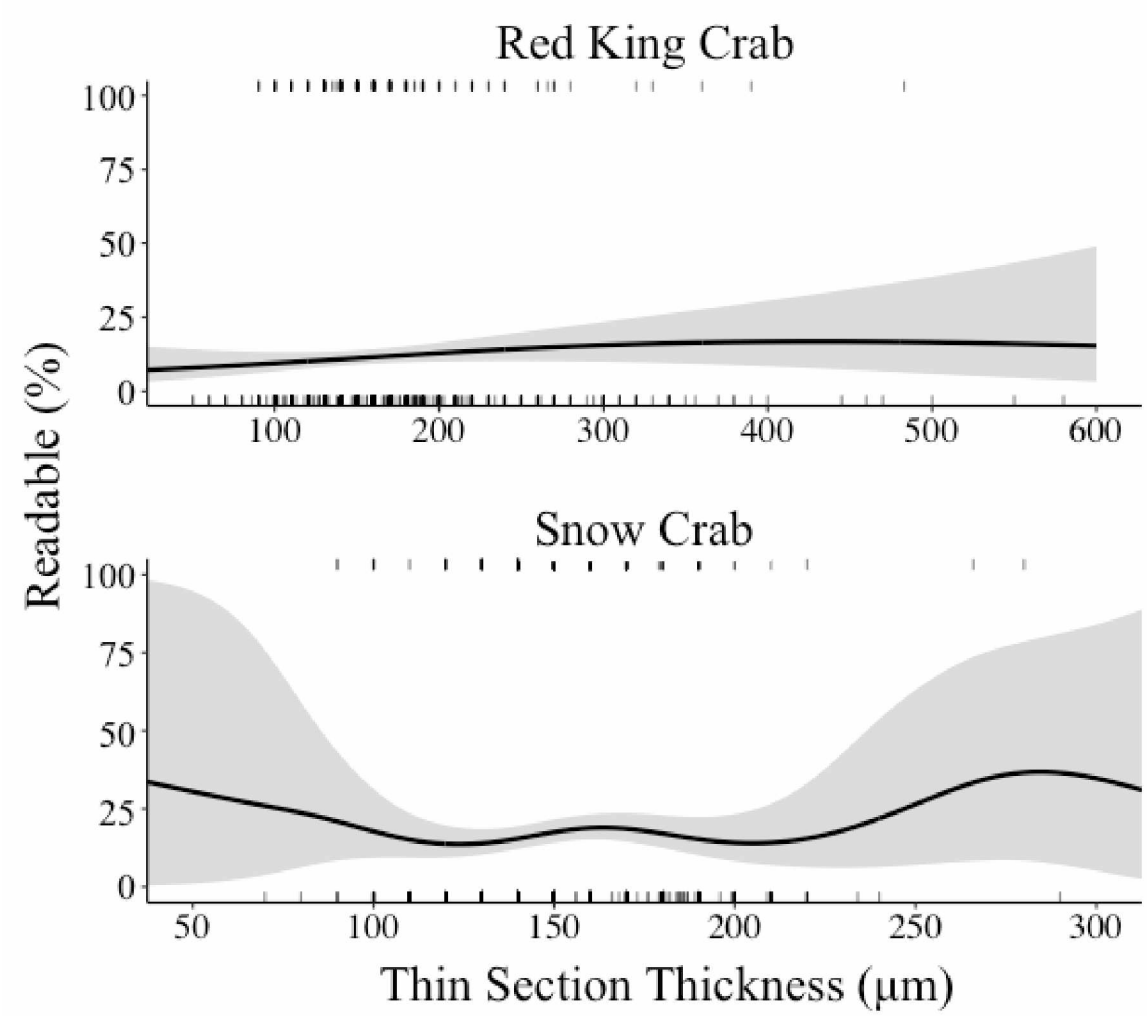

Figure 1.5. Crab thin section thickness readability. Predicted percent readable along the thickness range for thin sections (tick marks along the top and bottom) of red king crab and snow crab thin sections with 95% confidence intervals (shaded area) using a GAM. Tick marks are present for thin section thickness values collected and used in the analysis; the values vary due to precision saw performance.

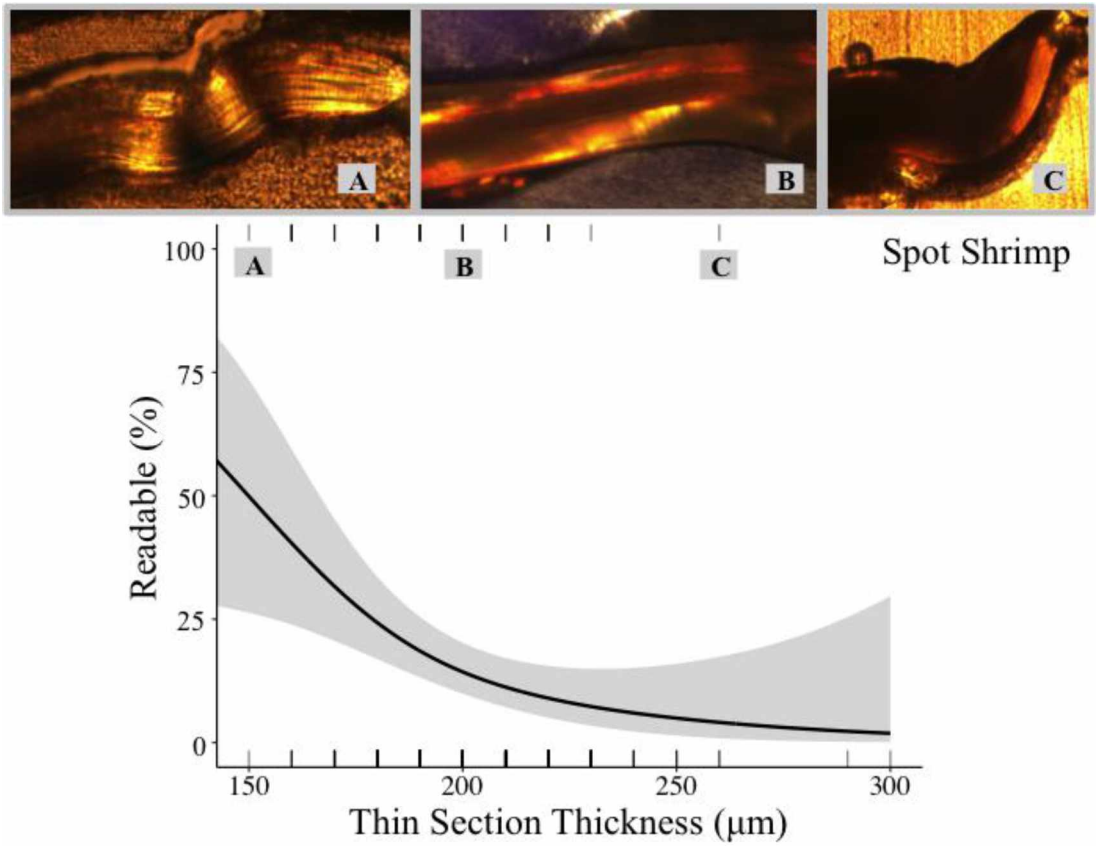

Figure 1.6. Shrimp thin section thickness readability. Predicted percent readable along the thickness range of spot shrimp thin sections with 95% confidence intervals (shaded area) using a GAM. Also shown are images of spot shrimp eyestalk thin sections under transmitted light corresponding to thicknesses of (A) 150 µm, (B) 200 µm, and (C) 260 µm. Tick marks are present for thin section thickness values collected and used in the analysis; the values vary due to precision of saw performance.

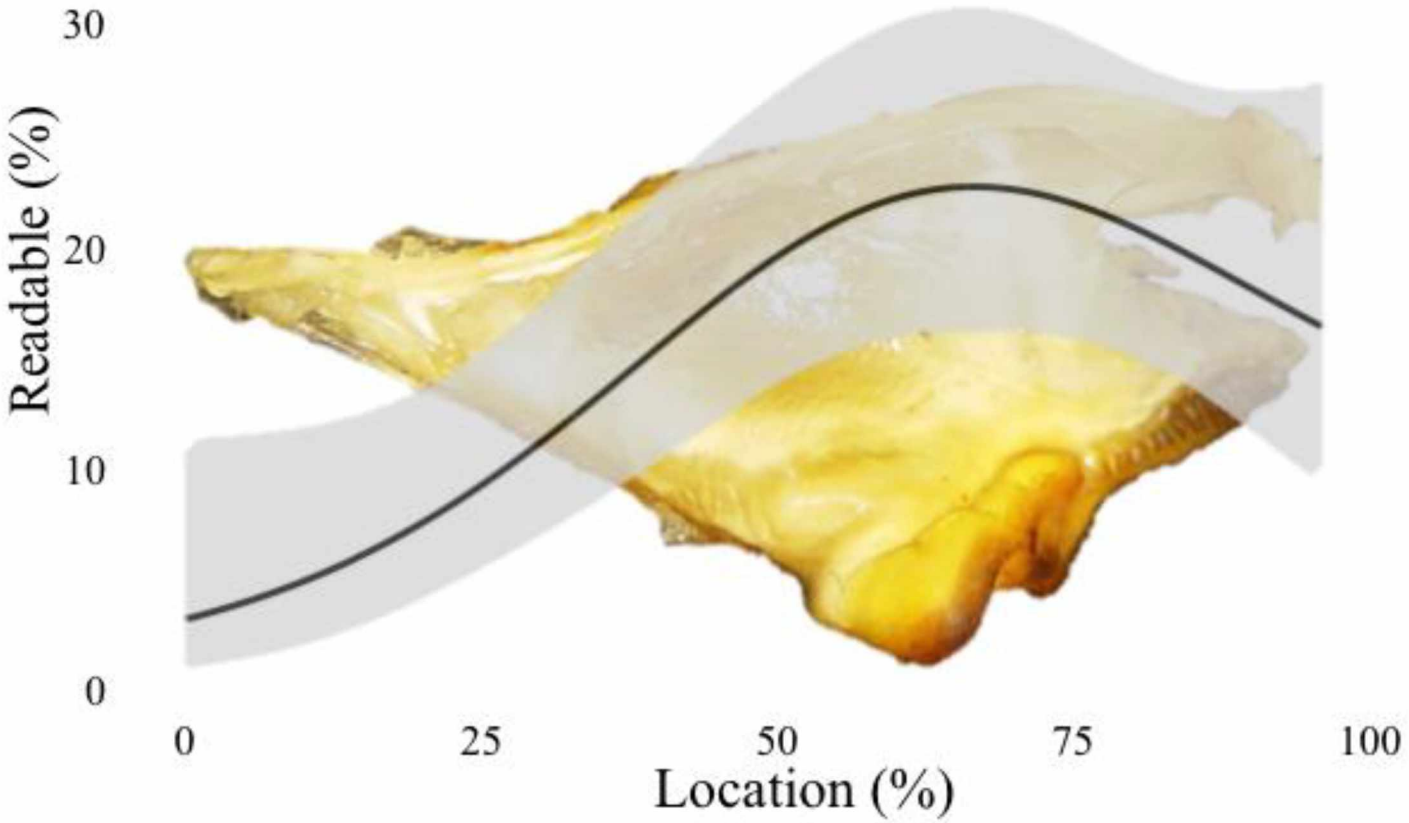

Figure 1.7. Red king crab zygocardiac readability. Predicted percent readable along the length of red king crab zygocardiacs with 95% confidence intervals (shaded area) using a GAM and pictured with an image of the structure aligned with corresponding locations.

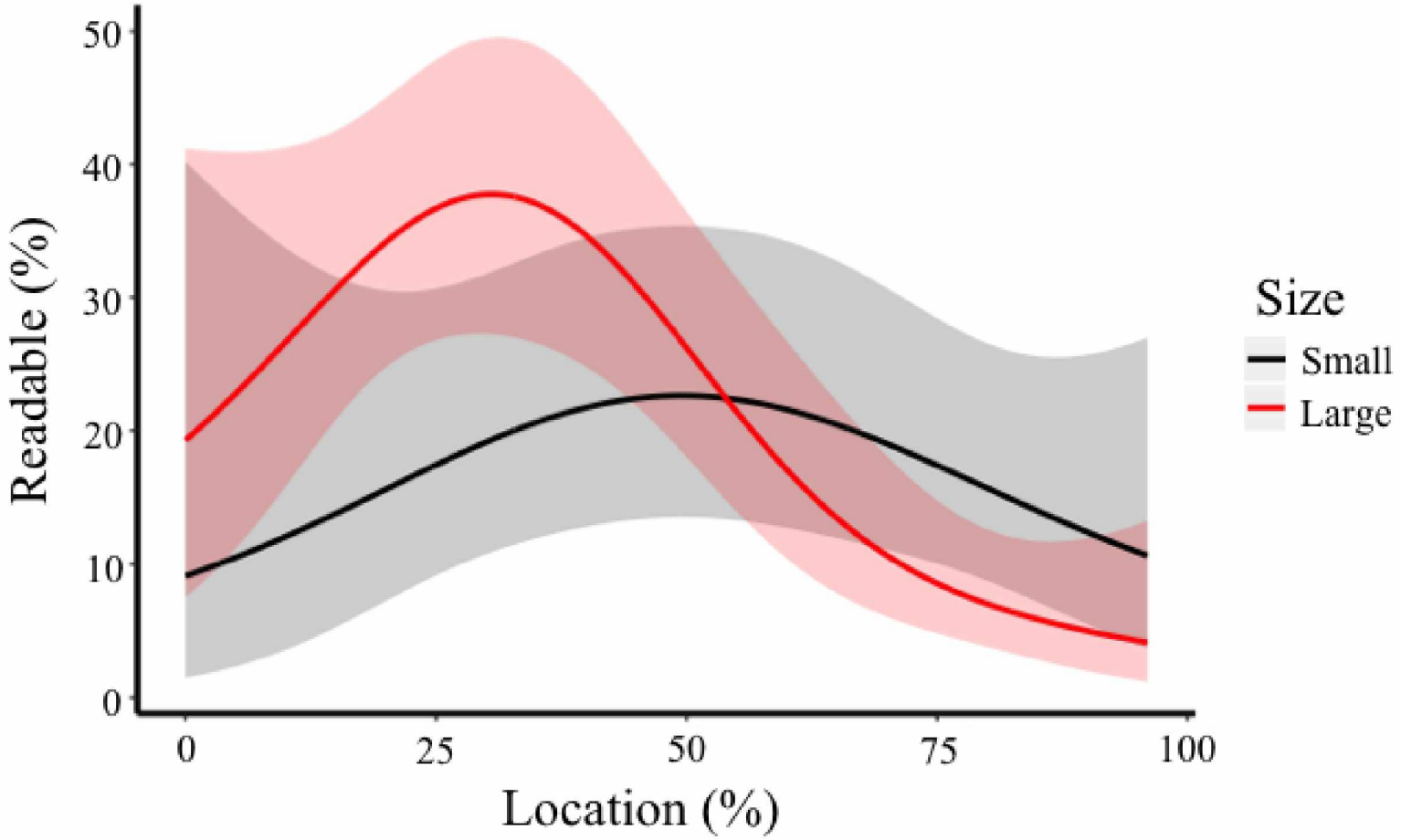

Figure 1.8. Large and small snow crab zygocardiac readability. Predicted percent readable along the length of snow crab zygocardiacs by large (carapace width 80-100 mm) and small (carapace width 40-60 mm) size classes with 95% confidence intervals (shaded area) using a GAM.

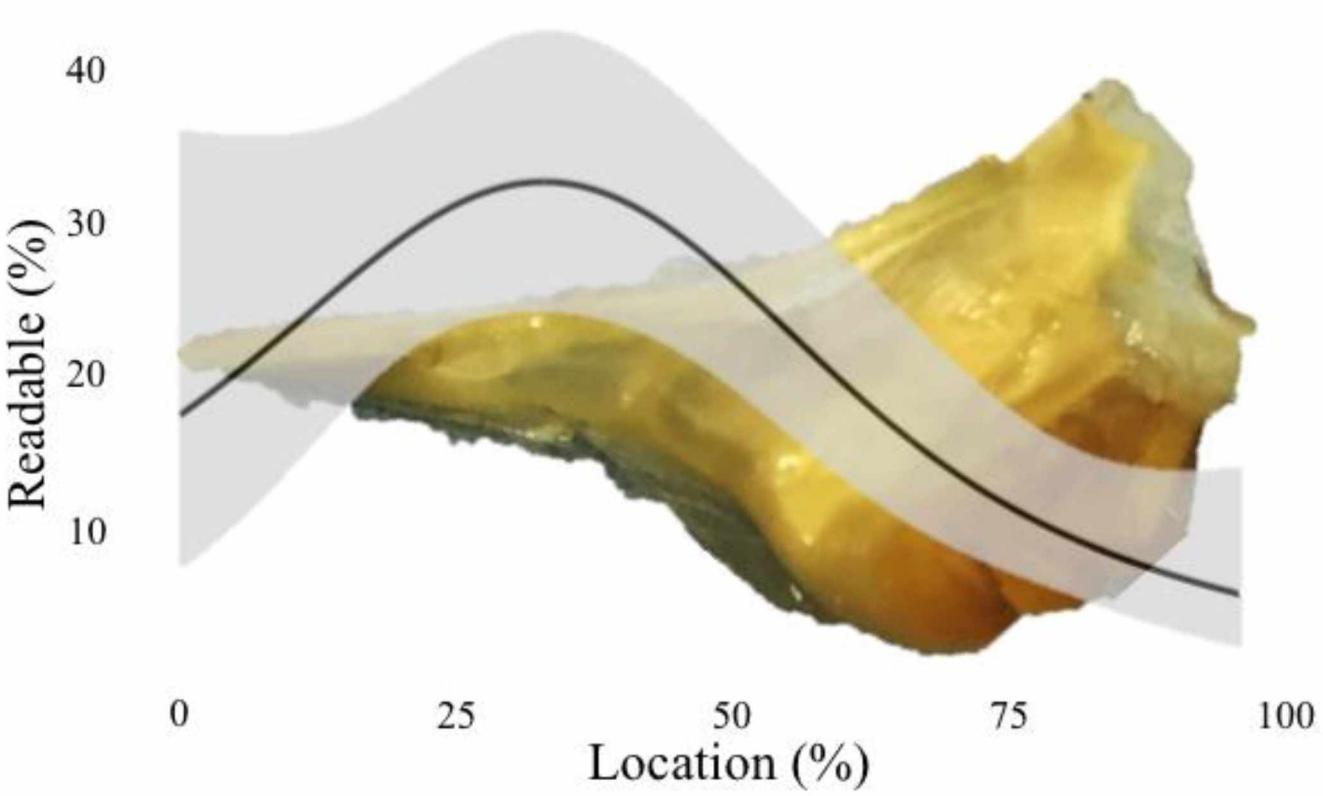

Figure 1.9. Snow crab zygocardiac readability. Predicted percent readable along the length of snow crab zygocardiacs with 95% confidence intervals (shaded area) using a GAMM and pictured with an image of the structure aligned with corresponding locations.

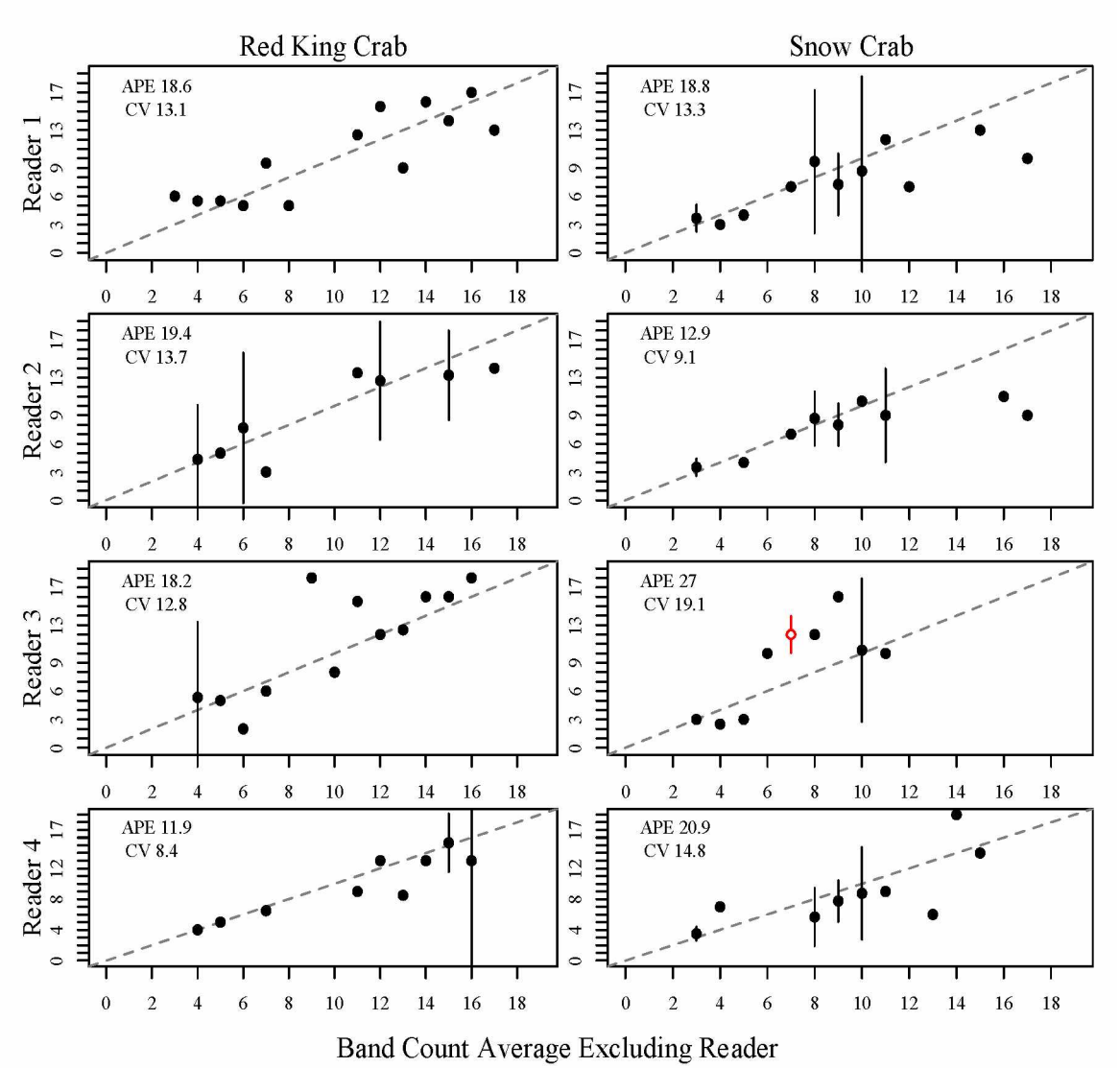

Figure 1.10. Independent red king and snow crab band count estimates for each of four readers against the average the other three readers for readable thin sections of various structures. Average percent error (APE) and coefficient of variation (CV) shown for each comparison. Vertical lines represent variance within for a given average band count estimate and the open point (with red confidence intervals) represents a band count estimate that differs significantly from the corresponding average estimate. The dashed line represents perfect agreement.

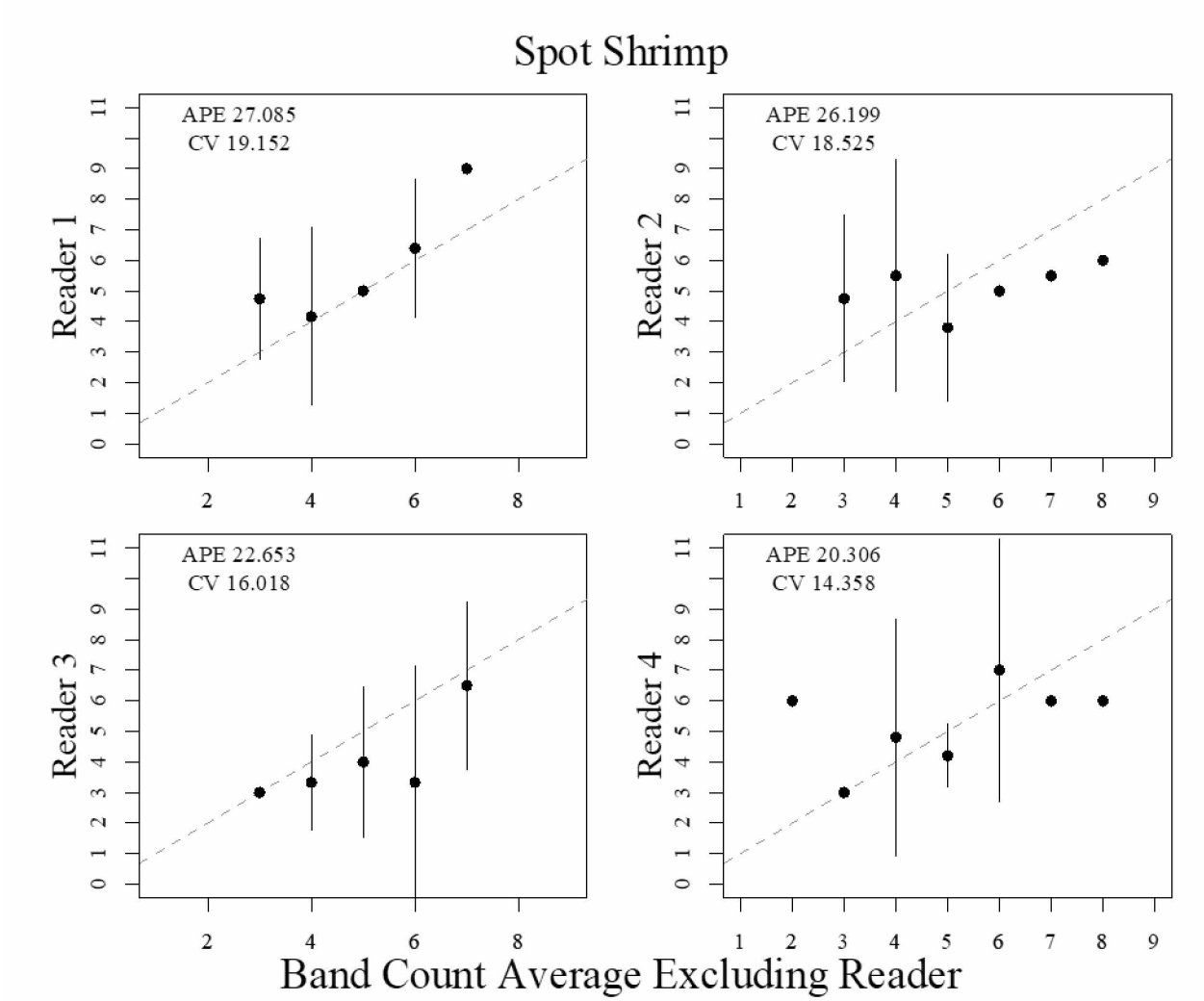

Figure 1.11. Independent spot shrimp band count estimates of each reader against the average the remaining three readers for readable thin sections of eyestalks thin sections. Average percent error (APE) and coefficient of variation (CV) displayed for each comparison. Vertical lines represent variance within for a given average band count estimate and the dashed line represents perfect agreement.

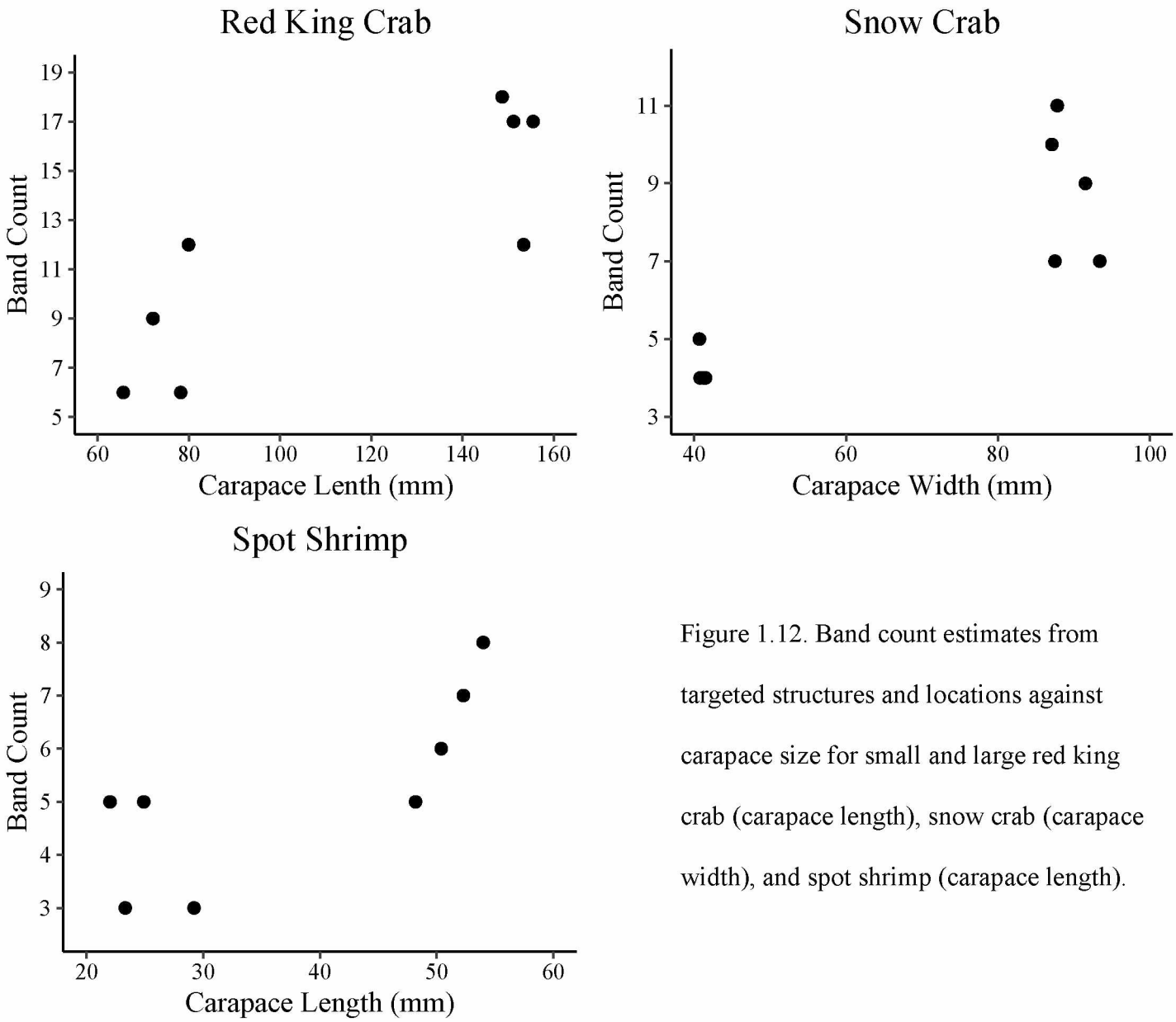

Figure 1.12. Band count estimates from targeted structures and locations against carapace size for small and large red king crab (carapace length), snow crab (carapace width), and spot shrimp (carapace length).

Tables

Table 1.1. Percent readable and unreadable thin sections for longitudinal (L) and transverse (T) section orientation by structure and species, including total count of thin sections used in the analyses for each orientation. An asterisk (*) denotes orientations alternate to previously established methods.

	Red king crab			Snow crab			Spot shrimp		
Structure	Orientation and Count	% Readable	% Unreadable	Orientation and Count	% Readable	% Unreadable	Orientation and Count	% Readable	% Unreadable
Eyestalk	L* (N=13)	4	96	L* (N=16)	23	77	L (N=11)	5	95
	T (N=15)	0	100	T (N=18)	33	67	T* (N=11)	5	95
Zygocardiac	L* (N=26)	0	100	L* (N=9)	0	100	NA	NA	NA
	T (N=33)	20	80	T (N=19)	19	81	NA	NA	NA
Pterocardiac	L* (N=23)	0	100	L* (5)	11	89	NA	NA	NA
	T (N=7)	0	100	T (15)	7	93	NA	NA	NA
Uromesocardiac	L (N=69, avg across specimens)	13	87	L (N=79, avg across specimens)	9	89	NA	NA	NA
	T* (N=20, 1 specimen)	16	84	T *(N=21, 1 specimen)	26	74	NA	NA	NA

Table 1.2. Akaike information criteria (AIC) model comparison results (rounded to whole numbers) for crab zygocardiac location (l), including degrees of freedom (df) and the difference between the lowest AIC value and the AIC value for each model (Δ AIC). N/A represents models for which convergence was not achieved.

	Snow Crab		Red King Crab	
Model $\alpha + \alpha_i + s_i(l)$	df	Δ AIC	df	Δ AIC
$\alpha + s(l)$	3	0	3	0
$\alpha + s_i(l)$	N/A	N/A	N/A	N/A
$\alpha + s_w(l)$	5	0	5	77
$\alpha + \alpha_i + s(l)$	4	28	4	42
$\alpha + w + s(l)$	4	2	4	82
$\alpha + \alpha_i + s_i(l)$	N/A	N/A	N/A	N/A
$\alpha + w + s_w(l)$	6	2	6	59

Table 1.3. Symmetry tests ($\chi 2$) results for independent band count estimates of four readers against the corresponding average for various structures of red king crab, snow crab, and spot shrimp. Degrees of freedom are displayed as df. Large p-values suggest that the estimates of readers were not systematically biased relative to the average of the remaining readers' estimates.

		Red king crab			Snow crab			Spot shrimp		
Reader		McNemar	Evans Hoenig	Bowker	McNemar	Evans Hoenig	Bowker	McNemar	Evans Hoenig	Bowker
	df	1	6	18	1	6	14	1	3	9
1	χ^2	0.4737	3.8	17	0.222	7.6	15.3	1.923	2.943	11
	p	0.4913	0.7037	0.5231	0.6374	0.2689	0.3558	0.1655	0.4005	0.2757
	df	1	5	16	1	6	10	1	4	11
2	χ^2	0.2222	7	18	1.3333	3.3333	10	0.6	4.2857	8.333
	p	0.6374	0.2206	0.3239	0.2482	0.7660	0.4405	0.4386	0.3687	0.6832
	df	1	6	14	1	8	15	1	4	11
3	χ^2	0.2857	4.666	14	3.5556	12.6666	14	7.142	8.666	14
	p	0.5930	0.5872	0.4497	0.0593	0.1238	0.5255	0.0075	0.06999	0.2390
	df	1	5	11	1	6	16	1	4	9
4	χ^2	0.333	5.1429	12	0.8889	3.2	18	0.692	4.866	7.667
	p	0.5637	0.3987	0.3636	0.3458	0.7834	0.3239	0.4054	0.3013	0.5681

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Chapter 2: Evaluation of a direct age determination method for terminally molted male snow crab *Chionoecetes opilio* (J.C. Fabricius 1788)[2]

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Abstract

Recent research suggests the utility of a direct method of age determination using calcified eyestalks or gastric mill (stomach teeth) ossicles for some crustacean species. Bipartite bands in the endocuticle, have been proposed to record annual growth (similar to fish scales or otoliths) that are independent of molting events. The method has been explored and refined for zygocardiac ossicles of the gastric mill from snow crab (*Chionoecetes opilio*, J.C Fabricius 1788). If proven effective, such a direct age estimation method could improve estimates of growth, mortality, recruitment, and age composition, which are needed to develop harvest strategies that optimize long-term economic yield while meeting crab resource conservation objectives. Although some studies show that the band counts correlate to other estimates of age for snow crab, little evidence exists to support that bands accumulate annually, independent of molt. Male snow crab undergo a terminal molt at maturity, after which they can survive for seven years or more, and shell condition, i.e. the degree of wear and epibionts on their exoskeleton, can be used as a proxy of time-elapsed since the terminal molt. We estimated band counts and endocuticle thickness from thin sections of the zygocardiac ossicle of terminally molted male snow crab across a range of shell conditions from a wild, fished stock. We found no differences in band counts (ANOVA, $P < 0.05$) or endocuticle thickness (ANOVA, $P < 0.05$) across shell conditions. Therefore, it does not appear that band counts can be used reliably for age estimation of this species, which undergoes a terminal molt.

Key Words: band counts, gastric mill, endocuticle, sclerochronology, snow crab

Introduction

Accurate age determination provides a measure of time and therefore plays a key role in research into growth and mortality rates and other parameters critical to successful fisheries management. Age estimates can be produced either directly from a hard structure in the animal or indirectly from other factors (e.g. changes in size composition) associated with age and growth. For many commercially important marine species, direct age estimates are produced from permanent structures such as vertebrae, shells, scales, and otoliths (ear bones), which grow continuously and record variability in growth throughout the lifespan (Jackson, 1994; Campana, 2001; Abele et al., 2009). However, crustaceans shed and replace their integument when they grow, resulting in loss of hard parts that might record age and/or growth. Consequently, crustacean age and growth estimation for stock assessment is largely derived from indirect indicators, such as carapace size, maturity, and shell condition (e.g. Zheng and Kruse, 2014). Indirect age estimation for crustaceans is based on mark-recapture, changes in size-at-instar, or size-at-maturity studies (Butler, 1961; Hartnoll, 2001; Fonseca et al., 2008), but the methods are limited in their application and do not reliably account for variables, such as temperature and food availability, which affect growth rates over time (Anger, 1984; McConaugha, 2017).

In contrast, direct methods of age determination are capable of identifying age and growth rate variability, which, combined with ancillary data, provide insights into variation among individuals and associated with environmental variability. Thus, development of direct age determination methods for crustaceans remains an elusive but high priority research objective. Recent developments in direct methods include the use of (1) lipofuscin, a naturally

occurring pigment shown to accumulate with age (Sheehy et al., 1994; Wahle and Tully, 1996; Allain et al., 2011; Pinchuk et al., 2016), (2) radionuclide activity in the exoskeleton (Foll et al., 1989), and (3) paired light and dark bands within the endocuticle of certain calcified structures (Leland et al., 2011; Kilada et al., 2012; Becker et al., 2018). In particular, the use of bands in eyestalks and gastric mill ossicles has been proposed as a potential method for three commercially important Alaska crustacean species including southern Tanner crab from the eastern Bering Sea (*Chionoecetes bairdi*, J.C Fabricius 1788) (Kilada et al., 2017).

Snow crab are brachyuran decapods that inhabit Atlantic Canada, Greenland and Norway/Russia (Barents Sea) and North Pacific oceans. The eastern Bering Sea snow crab stock supports the most valuable commercial crab fishery in Alaska (NMFS, 2017), but has historically faced overfishing. Key population dynamics parameters including natural mortality remain poorly known (Zheng, 2003) resulting in conservative harvest levels (Szuwalski, 2018). Male snow crab undergo a terminal molt at maturity (Conan and Comeau, 1986; Tamone et al., 2005), after which they can survive up to seven years or more (Fonseca et al., 2008). The degree of wear and epibionts on their exoskeleton (i.e. shell condition) can be used as an approximate index of time elapsed since the terminal molt (Fonseca et al., 2008).

Several studies have shown relationships between band count and carapace size for snow crab (Kilada et al., 2012; Rebert et al., unpublished) and other crustaceans (Kilada et al., 2012; Leland et al., 2015). However, there is a lack of rigorous evidence (e.g. validation) that the observed bands represent annual growth increments for snow crab. Annual increment (band) formation would parsimoniously presume that gastric mill ossicles continue to grow in the

absence of molting, contrary to the accepted paradigm of crustacean growth (Crook et al., 2018). The objective of this study is to observe and examine differences in endocuticle thickness and band counts across shell condition groups of terminally molted snow crab representing, on average, several years of elapsed time without molting. If the endocuticle region thickens and accumulates bands with older shell condition groups, which broadly reflect increasing post-molt age, this would provide evidence for potential value of the direct age determination method from a wild, fished stock.

Materials and Methods

Specimen Collection

A total of 60 large (between 87.1–140.7 mm carapace width) male snow crab of three shell condition classes (new, old, and very old) as described in Ernst et al. (2005) (n = 20 per group) were collected during the National Marine Fisheries Service crab and groundfish bottom trawl survey from southeastern Bering Sea shelf in July 2016 (Daly et al., 2016). All specimens were confirmed to be mature and terminally molted based on the chela height to carapace width ratio criterion > 0.2 (Tamone et al., 2007). Specimens were frozen at sea and sent to the ADF&G Mark, Tag, Age Lab, Age Determination Unit (ADU) in Juneau, Alaska. In the ADU laboratory, carapace width and shell condition data were collected, and gastric mill ossicles were removed, cleaned and preserved in a 70:3:27 mixture of ethanol, glycerol, and distilled water for ≥ week prior to being processed for the experiment.

Structure Processing

Zygocardiac ossicles were processed following the methods recommended for snow crab by Rebert et al. (unpublished). The left zygocardiac ossicles (or the right if the left was damaged) were cleaned and their lengths were measured with digital calipers (± 0.01 mm) (Fig. 2.1). The zygocardiacs were then embedded in System Three® cold cure epoxy and thin-sectioned transversely, within 20-60% of the structure's total length from posterior to anterior end (Fig. 2.1) with a Buehler® IsoMet™ 5000, high-speed diamond blade precision saw, which was programmed to cut each section at 180 μm. Thin sections were produced, mounted on petrographic slides, and evaluated with a Leica DM 2000 compound microscope using transmitted and reflected light, wetted with tap water, and under magnification between 100x and 200x. Thin section readability was based on presence and clarity of banding patterns and determined subjectively as either readable or unreadable. Only readable thin sections were used to produce band counts while both readable and unreadable sections were used for endocuticle measurements.

Band Count

The number of paired (bipartite) bands were estimated from readable sections for each specimen using criteria developed and tested in Rebert et al. (unpublished). To ensure quality of band count estimates, readable thin-sections were initially examined independently by two readers. Then, differences in reader estimates were evaluated by visual and graphical analyses with age-bias plots and age difference plots, using diagnosis of paired age agreement methods described by McBride et al. (2005) and the FSA package in R (Ogle, 2018). In addition, average percent error (APE) (Kimura and Anderl, 2005; Cailliet et al., 2006) and Chang's coefficient of variation (CV) (Campana et al., 1995) were used as indices of band count precision between readers. Any

band count estimate discrepancy between the readers for the same thin-section was resolved by a third ("determinator") estimate produced by subsequent discussion resulting in mutual agreement by the two readers.

Statistically significant differences ($\alpha = 0.05$) in band count among shell condition groups were evaluated using an ANOVA with shell condition group as a fixed effect:

$$B_{ij} = \mu + S_j + \varepsilon_{ij},$$

where B is band count estimate for specimen i, μ is the average band count, and S is the difference of each shell condition j from the mean. In addition, a relationship between band count and specimen size (carapace width) was similarly evaluated using linear regression:

$$B_i = \beta_0 + \beta_1 W_i + \varepsilon_i,$$

where B is band count estimate for specimen i, W is the specimen size, β_0 is the intercept, and β_1 is the regression coefficient for specimen size.

Endocuticle Measurements

For each specimen, the first thin section within the targeted area of the zygocardiac (at 20% of the structure length, Fig. 2.1) was imaged using a Leica DFC450 Camera and measured using Media Cybernetics Image-Pro® Premier 9.2 analysis software. The endocuticle was measured from the exocuticle to the hypocuticle in a standardized location perpendicular to the endo-exocuticle boundary (Fig. 2.2). This measurement runs along the same thin section axis used for band count estimates. Thin sections that were damaged were excluded from analysis. To ensure

data quality, two measurements were taken independently and the average of the two measurements was used in the analysis.

Statistically significant differences ($\alpha = 0.05$) in endocuticle measurements among groups were evaluated using an ANOVA with shell condition group as a fixed effect:

$$T_{ij} = \mu + S_j + \varepsilon_{ij},$$

where T is endocuticle thickness for specimen i, μ is average endocuticle thickness, and S is the difference of each shell condition j from the mean.

Results

Structure Processing and Band Count

Processing structures from dissection to band count production required approximately 40 hours of time for the 60 specimens. Upon completion of sectioning the zygocardiacs, a total of 864 thin sections were produced and evaluated for readability. An average of 14 thin sections were taken from each zygocardiac. Despite using refined methods to process the zygocardiac, we were unable to produce thin sections with clear banding patterns for all specimens, therefore our evaluation was limited to smaller sample sizes than intended. At least one thin section was found to be readable for 16 new, 17 old, and 21 very old shell specimens (54 out of the 60 total specimens), and those 54 specimens were used for band count estimation.

Comparisons between band count estimates of independent readers produced four specimens with exact agreement and 50 with discrepancies; therefore, determinator reads were

produced for the 50 specimens. Determinator band count estimates for all specimens ranged from 6 to 16. Results from bias plots (Fig. 2.3) suggested differences in band count estimates between the two age readers; calculated APE (10-14%) and CV (7.1) (Campana, 2001). For new, old, and very old shell conditions, average determinator band counts for each group were 11.1, 10.2, and 10.9 respectively (Fig. 2.4), and determinator band count estimates did not significantly differ by shell condition (ANOVA: df = 2, F = 0.5657, P = 0.5715; Fig. 2.4). Because band count estimates differed between age readers, we chose to run a similar ANOVA using both datasets. Results from those analyses also showed that band count estimates did not significantly differ by shell condition (Reader1 ANOVA: df = 2, F = 0.1109, P = 0.8953; Reader2 ANOVA: df = 2, F = 0.6113, P = 0.5466). In addition, the linear regression results showed that specimen size did not predict band count for any of the three estimates (determinator F = 0.8502, P = 0.3608; Reader1 F = 0.7926, P = 0.3774; Reader2 F = 1.191, P = 0.28).

Endocuticle Measurements

Due to damaged sections at the standard location (20% of structure length), endocuticle measurements were produced for 15 new, 19 old, and 22 very old shell specimens. Measurement values ranged from 0.16 to 0.69 mm. For new, old, and very old shell conditions, average endocuticle measurements were 0.41, 0.43, and 0.37 mm, respectively (Fig. 2.5). Endocuticle measurements did not significantly differ among shell condition groups (ANOVA df = 2, F = 2.3173, P = 0.1086; Fig. 2.5).

Discussion

This study investigated the possibility that the endocuticle of male snow crab continues to grow, increasing in thickness and forming growth bands, after the terminal molt. Instead, we found evidence that endocuticle thickness and band counts are similar across shell conditions, suggesting that the endocuticle does not thicken or form growth bands after the terminal molt.

Optimizing methods from Rebert et al. (unpublished) yielded two improvements in producing band counts. Firstly, we saw an improved efficiency in processing structures from dissection to band count, producing counts from readable sections for double the specimens in nearly a quarter of the time (Rebert et al., unpublished). Secondly, while the results for average percent error (APE) and CV between readers are considered imprecise for age data (Campana, 2001), band count precision appeared to improve in this study, with a lower maximum average percent error (APE) between reader and determinator band count estimates (10% and 14.5%, respectively) in comparison to the maximum APE (27%) previously reported between readers in Rebert et al. (unpublished).

We found no evidence that band counts are related to variation in shell condition which is known to broadly index the time after terminal molt for male snow crab. However, these results do not exclude the possibility that the banding patterns observed in the gastric mill ossicles could represent growth history related that vary with size/age prior to the terminal molt. Results from this study did not support that counts are related to size. However, using similar methods Rebert et al. (unpublished), which included much smaller male snow crab, found that that large male snow crab had more bands than small snow crab. The difference in results in this study may be due to the limiting of our samples to large crab only. Several studies have also argued that band

counts may not be related to growth histories since: 1) All or a portion of the gastric mill ossicles are not retained through the molting cycle in at least some species (Sheridan et al., 2015; Vatcher et al., 2015; Sheridan et al., 2016; Becker et al., 2018); 2) There is a lack of definitive evidence of continuous growth of the gastric mill ossicles which violates a key tenet for evaluation of structures used for age determination (Crook et al., 2018); and 3) The banding patterns observed in the endocuticle may be features of the endocuticle microstructure, independent of age (Becker et al., 2018).

The average band count estimates observed in this study, while they did not increase with shell condition, would be reasonable estimates of current knowledge of snow crab age-at-size estimated from growth and analysis of size-frequency distributions. Male snow crab are estimated to be between 4.5 years (Robichaud et al., 1989) and as many as 9.5 years (Watson, 1971) post-hatch when they undergo terminal molt. According to Sainte-Marie et al. (1995), male snow crab > 79.79 mm carapace width is modeled as being between 7.7 and 10.7 years old and as having molted at least 11 times. In addition, molt increments can differ for male snow crab that skip molt (Hébert et al., 2002). Our band count estimates ranged from 6 to 16; values that are not unreasonable estimates for age or molt frequency. However, specimens from each shell condition group have estimates ranging from 7 to 15, meaning new shell terminally molted crab have up to 15 bands, which is unreasonably high for either molt frequency or age.

Similar to band counts, our endocuticle measurements for very old shell crab are slightly smaller than for the other shell conditions (Fig. 2.5). Fonseca et al. (2008) observed shell degradation subsequent to this portion of the life cycle, which is consistent with the apparent

reduction in endocuticle thickness for very old shell snow crab. Similarly, Crook et al., 2018, found zygocardiac ossicle diameters were higher in supposed 1-year-old than 2-year-old Giant mud crab (*Scylla serrata*). If the cuticle is degrading with time, any growth information contained within it will degrade, as well. Our endocuticle measurement results indicate it is unlikely that accurate chronological age information can be reliably derived from the zygocardiac ossicle and other proposed structures of the gastric mill from terminally molted snow crab (Rebert et al., unpublished).

In general, age structures possess an origin, around which material is deposited (Campana and Neilson, 1985). One possible source of bias in band count determination would occur if the region of a structure that is sectioned does not contain the origin and the entire growth history. If the zygocardiac contains a growth origin, band counts may vary at different locations along the structure (Crook et al., 2018). For snow crab, we produced standardized thin sections from specific areas of the zygocardiac for band counts and endocuticle measurements following the recommendations of Rebert et al. (unpublished). Anecdotally, we did not find evidence of differences between sections and no studies have shown potential variation across the zygocardiac for snow crab. However, our thin sections were standardized at the target location and so this effect would not be biased in comparing band count across different shell conditions.

Even though this study does not support the use of band count estimates as an indicator of age for terminally molted snow crab, the technique may be useful as a direct or indirect estimate of chronological age for species with regular, annual growth. Further evaluation of this technique and increased band count precision may improve results, but objective measurements of the

endocuticle corroborate band count results. Shelton and Chapman (1986) demonstrated an alternative method to producing band counts using pieces of cuticle injected into crab to induce production of "living tags". These structures may be useful in recording time at liberty and as an alternative to external tags that may not be retained through molt. However, we do not recommend using current endocuticle band count methods to produce age estimates for this species.

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Figures

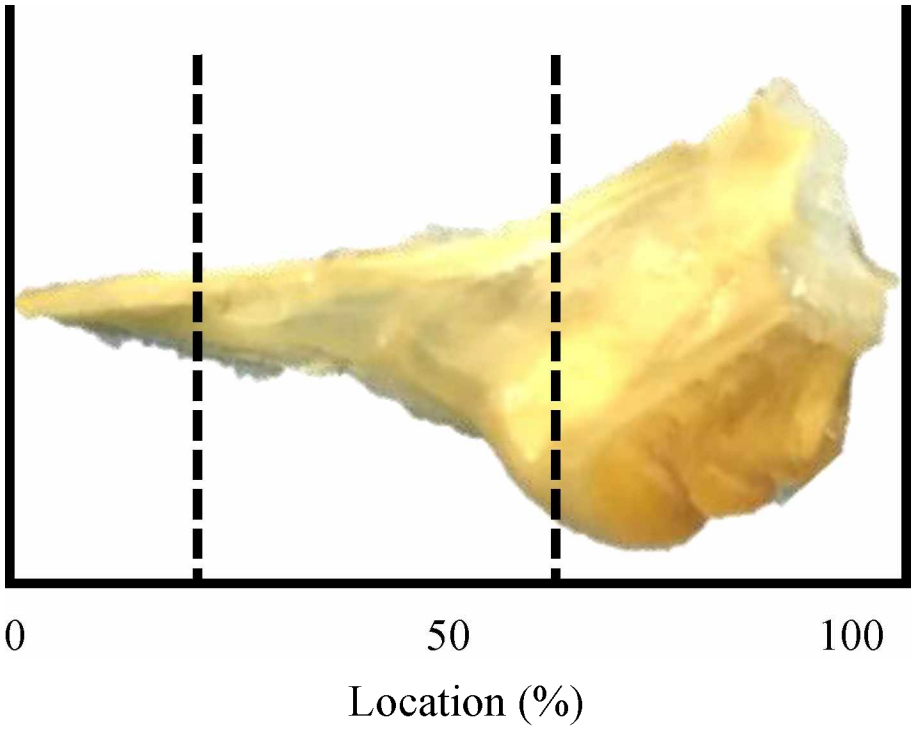

Figure 2.1. Snow crab zygocardiac length from 0% (posterior) to 100% (anterior). The targeted sectioning area, between 20-60%, is illustrated with dashed lines. Endocuticle measurements were derived from thin sections taken at 20%.

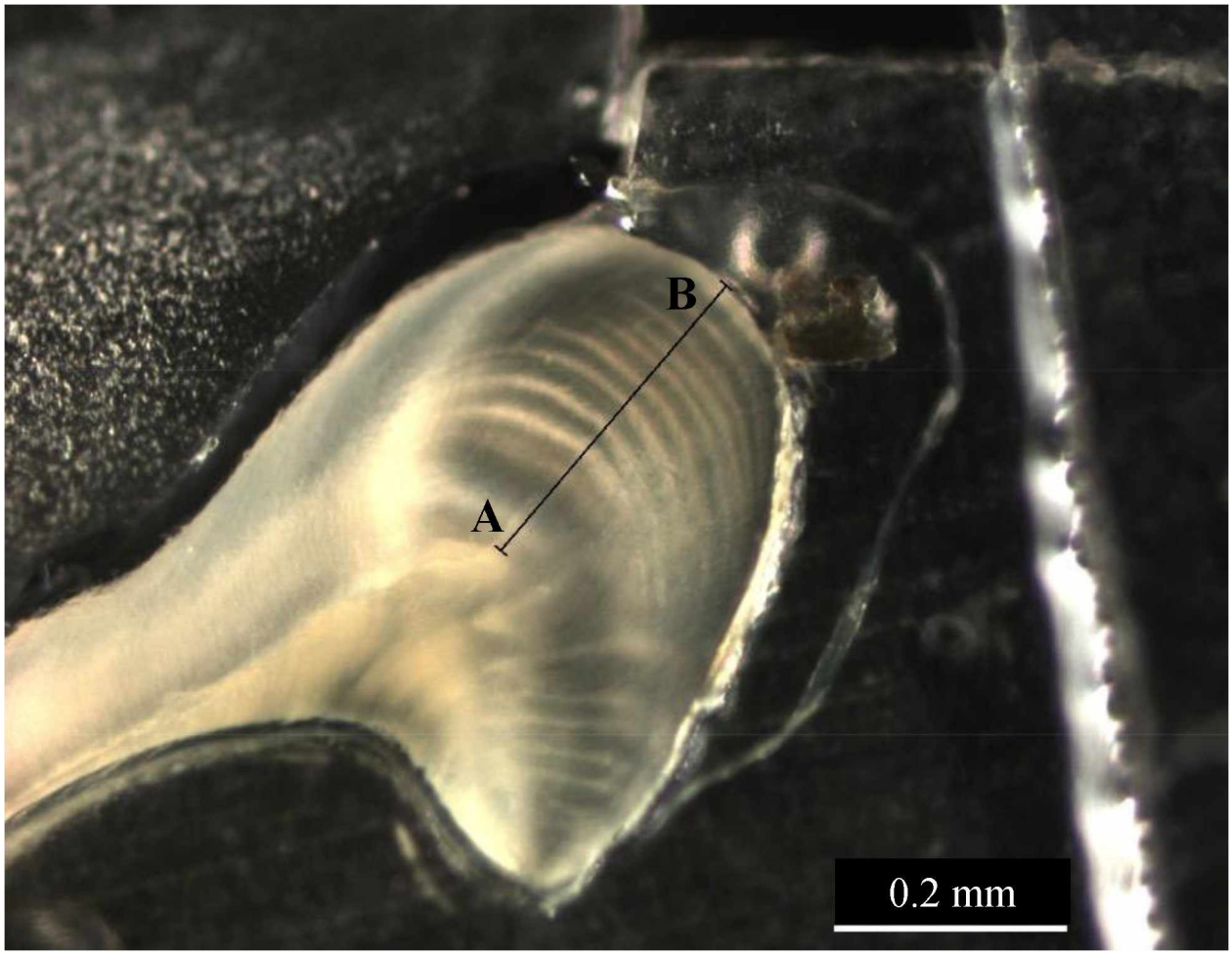

Figure 2.2. Standardized zygocardiac thin section from a terminally molted male snow crab with the endocuticle measurement from the endo-exocuticular boundary (A) to the hypocuticle (B).

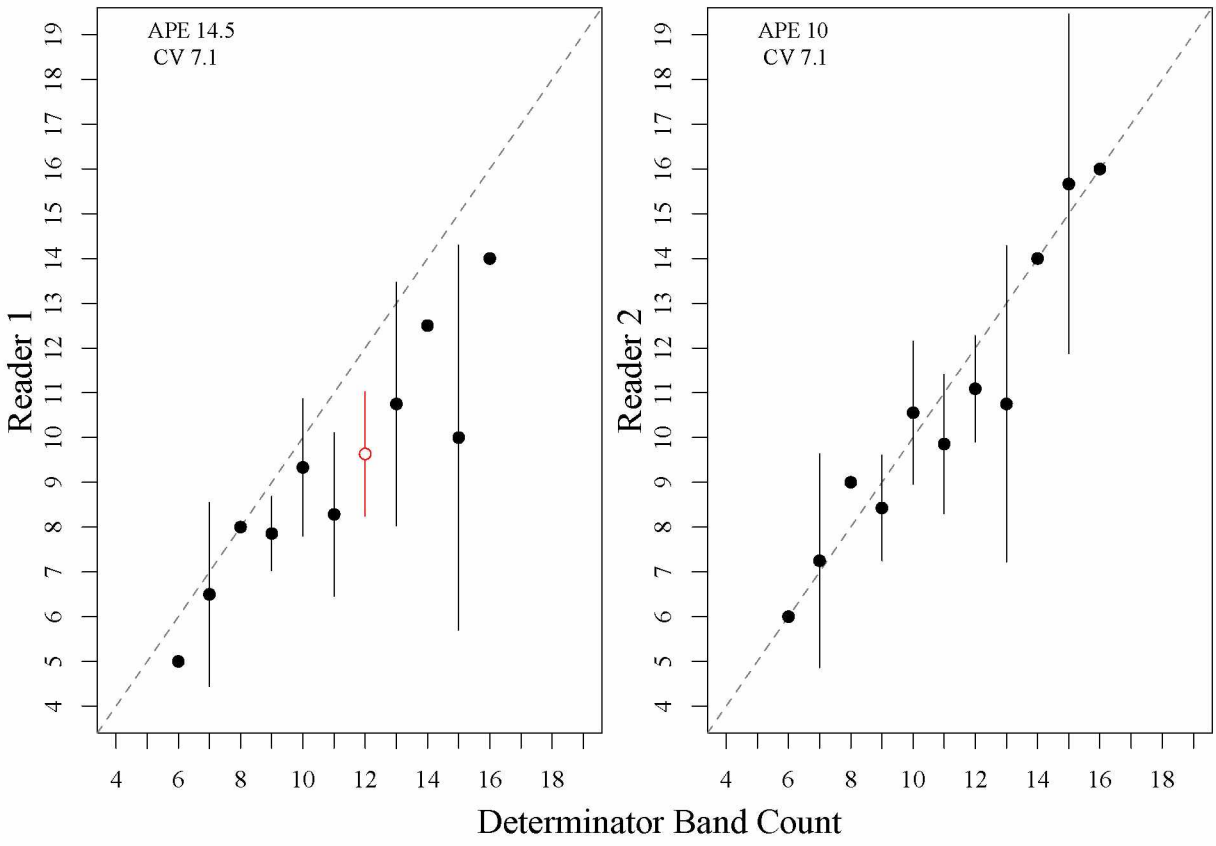

Figure 2.3. Independent band count estimates for each reader against determinator estimates for readable thin sections from 20-60% of the zygocardiac length for 54 terminally molted snow crab. Average percent error (APE) and coefficient of variation (CV) are shown for each comparison. Vertical lines represent variance within for a given determinator band count estimate and the open red point (and confidence intervals) represents a band count estimate that differs significantly from the corresponding average estimate. The dashed line represents perfect agreement.

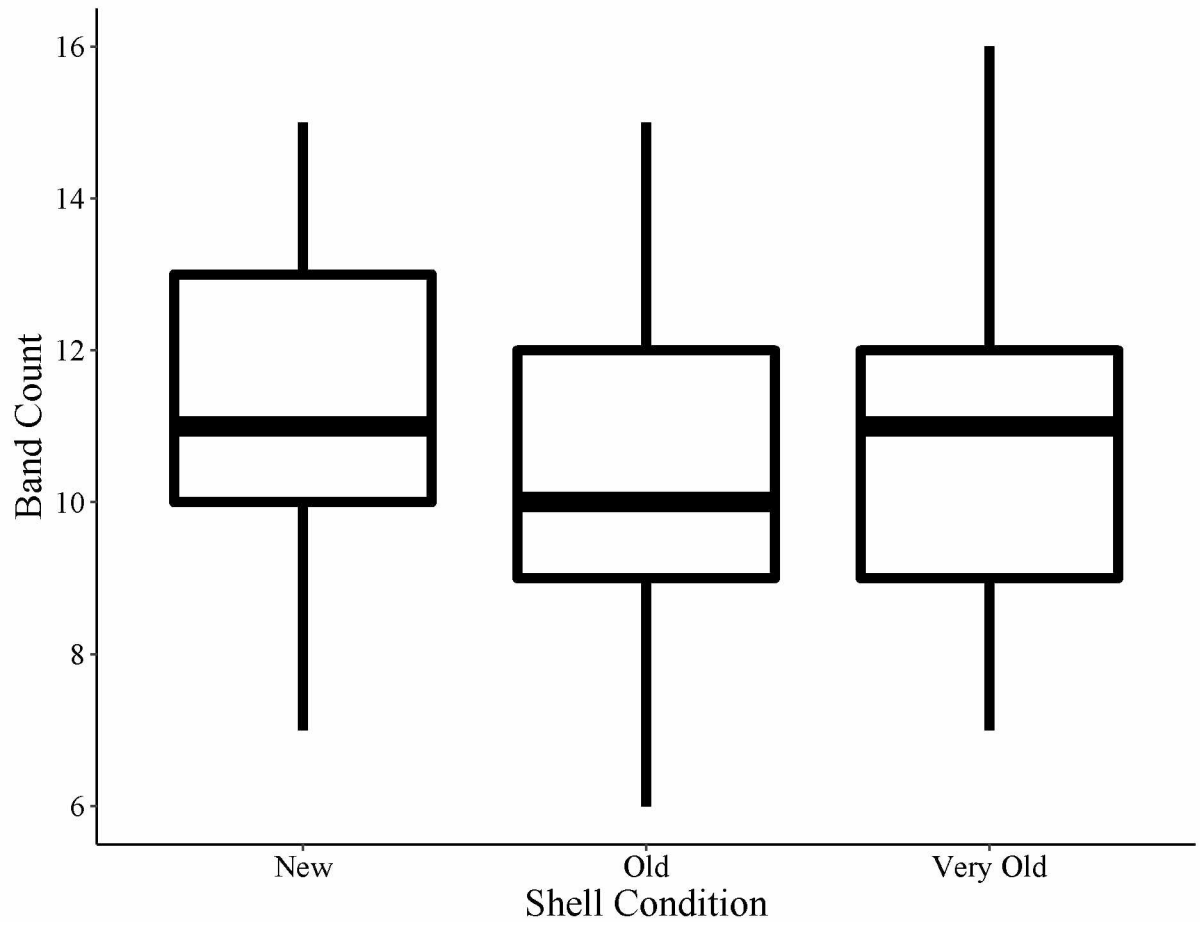

Figure 2.4. Final band count estimates (determinator reads) by shell condition. Boxes consist of the first quartile (bottom horizontal line), median (bold horizontal line), and third quartile (top horizontal line). Vertical lines are spread from the minimum to the maximum band count estimates for each shell condition group.

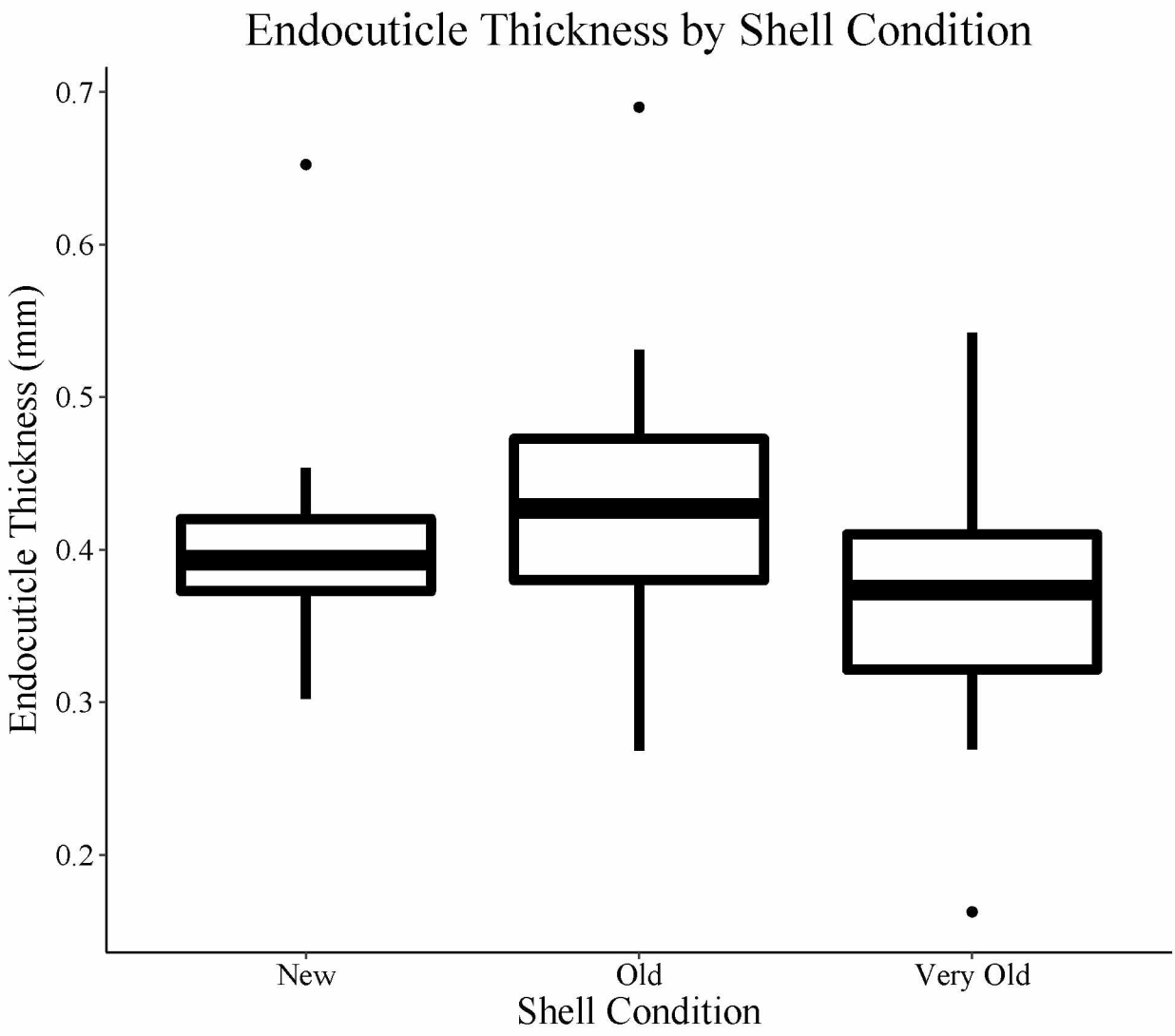

Figure 2.5. Endocuticle measurements across shell condition. Boxes consist of the first quartile (bottom horizontal line), median (bold horizontal line), and third quartile (top horizontal line). Vertical lines are spread from the minimum to the maximum endocuticle thickness measurements for each shell condition group. Points indicate outlying measurements.

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General Conclusions

Crustaceans are an integral part of economic and environmental stability of global marine resources. Within the United States, Alaska produces highly valuable commercial crustacean fisheries (FAO, 2016; Garber-Yonts and Lee, 2017; Smith and Gray, 2017), with direct connections to humans through activities such as personal use harvesting, commercial fishing, tourism, and research. Beyond food supply, the industry involved in Alaska's crustacean fisheries enhances jobs, technological advancements, and domestic and foreign commerce (Seung et al., 2015). Yet, lack of accurate crustacean growth and age data limits the ability to manage stocks and understand stock behavior, especially under varying conditions.

Climate changes and increasing atmospheric and oceanic carbon dioxide concentrations present a suite of challenges for natural resource management. According to the current reported ocean acidification mortality effects, catch and profits from the southern Tanner crab fishery in the eastern Bering Sea are expected to decrease more than 50% by 2036 (Punt et al., 2015). Projected pH values for the next century suggest that ocean acidification will continue to have negative effects on commercially important Alaska crustaceans (Bechmann et al., 2011; Long et al., 2013; Swiney et al., 2015). The likely population declines will be part of a greater cascade affecting large trophic systems and dynamics. Because accurate age determination can be used to assess changes in mortality and growth, development of such methods would be imperative for evaluating long-term environmental and human effects and possible resilience throughout the life cycle.

Results from Chapter 1 of this thesis indicate optimal methods for continued evaluation of recently proposed direct age determination methods for three commercially important Alaska crustaceans: red king crab, snow crab, and spot shrimp. In addition, we observed more bands in structures from larger individuals than from smaller individuals of each species; however, band counts are limited by small sample sizes for specimens of each species and large discrepancies in pattern interpretation. Though band count precision was suboptimal, the bands may indicate growth and the standardized methods that were developed will allow for efficient and robust comparison in future studies.

Chapter 2 assessed endocuticle thickness and band among male snow crab of varying shell condition and determined that bands nor the endocuticle evidently accumulate over time in the absence of molting for snow crab. Processing efficiency was improved and band count estimates for some of the specimens were reasonable when compared to current knowledge of cumulative molts or age-at-size, but estimates for other specimens were unreasonably high for molt frequency or age. Similar to Chapter 1, band count precision between readers was suboptimal. Chapter 2 results suggest that the methods, as tested, cannot be applied for age estimation of terminally molted snow crab. but may be useful as a direct or indirect estimate of chronological age for species with regular, annual growth.

The potential direct age determination for crustaceans uses bands within the endocuticle of calcified structures. Validation of the methods has been described by application of chemical tags to the crustacean endocuticle to demonstrate retention through the molting process (Kilada et al., 2012; Leland et al., 2015). However, studies have shown that the structures in which bands

were observed and evaluated, including the endocuticle, can also be lost during the molting cycle (Becker et al., 2018; Sheridan et al., 2015;). Accurate crustacean age data are an urgent research need for improved management, but failure to sufficiently validate age determination methods can lead to mismanagement and over- or under-exploitation of stocks (Beamish and McFarlane 1983; Campana, 2001).

This thesis produced species-specific, standardized procedures for preparing readable thin sections from calcified structures for three commercially important Alaska species. However, more evidence is needed to validate that banding patterns are direct and accurate expressions of age. Consequently, we recognize the limitations and do not recommend the direct age determination methods using banding patterns within gastric mill ossicles or eyestalks for red king crab, snow crab, and spot shrimp at the present time.

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